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Atividade biológica e efeito antioxidante de lipossomas obtido de extrato de folhas de *Protium heptaphyllum* no tratamento da obesidade e mutagênese induzidos por ciclofosfamida

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— Autor desconhecido

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RESUMO

O estudo de substâncias naturais tem despertado grande interesse na comunidade científica, especialmente devido aos seus potenciais benefícios para a saúde humana. Entre essas substâncias, *Protium heptaphyllum* (*P. heptaphyllum*), popularmente conhecido como breu-branco, destaca-se por suas propriedades bioativas, com ênfase nas folhas, que são ricas em compostos como flavonoides e triterpenos. Esses componentes têm mostrado efeitos promissores no combate a processos inflamatórios, antioxidantes e no manejo de doenças metabólicas, como a obesidade — uma condição que envolve mecanismos complexos de estresse oxidativo. Uma das abordagens mais inovadoras para potencializar os efeitos terapêuticos de compostos bioativos envolve o uso de lipossomas, sistemas de liberação que aumentam a biodisponibilidade e a estabilidade de substâncias. Lipossomas são vesículas esféricas compostas por uma ou mais camadas de fosfolipídios, capazes de encapsular compostos como o extrato de *P. heptaphyllum*, maximizando sua eficácia no combate aos danos oxidativos e inflamatórios. A obesidade é frequentemente associada ao estresse oxidativo, um desequilíbrio entre a produção de espécies reativas de oxigênio (EROs) e a capacidade antioxidante do organismo. Esse estresse desempenha um papel central no desenvolvimento de várias comorbidades, como dislipidemias, resistência à insulina e doenças cardiovasculares. Além disso, o uso de ciclofosfamida, um agente imunossupressor, em modelos experimentais pode intensificar os efeitos oxidativos e inflamatórios, criando um cenário ideal para testar a eficácia de substâncias naturais com propriedades antioxidantes e anti-inflamatórias. Nos estudos analisados, além de uma revisão literária sobre a *P. heptaphyllum* foram utilizados modelos experimentais que induziram obesidade em animais por meio de dieta hipercalórica, seguidos de tratamento com a fração acetato de etila do extrato de folhas de *P. heptaphyllum* encapsulado em lipossomas. E também, foi investigada a ação quimioprotetora da fração acetato de etila do extrato de *P. heptaphyllum*, encapsulado em lipossomas, contra os danos oxidativos causados pela ciclofosfamida, permitindo uma avaliação abrangente dos efeitos protetores do tratamento. Os resultados desses estudos indicam que o uso de *P. heptaphyllum*, especialmente na forma encapsulada em lipossomas, pode desempenhar um papel significativo na atenuação dos efeitos do estresse oxidativo, inflamação e distúrbios metabólicos associados à obesidade e ao estresse induzido pela ciclofosfamida. A combinação das ações antioxidantes e anti-inflamatórias dos flavonoides, potencializadas pelos lipossomas, revela uma estratégia promissora no combate aos efeitos negativos de condições metabólicas e imunossupressoras.

Palavras-chave: tratamento fitoterápico, flavonoide, estresse oxidativo.

ABSTRACT

The study of natural substances has aroused great interest in the scientific community, especially due to their potential benefits for human health. Among these substances, *Protium heptaphyllum* (*P. heptaphyllum*), popularly known as breu-branco, stands out for its bioactive properties, with emphasis on the leaves, which are rich in compounds such as flavonoids and triterpenes. These components have shown promising effects in combating inflammatory processes, antioxidants and in the management of metabolic diseases, such as obesity — a condition that involves complex mechanisms of oxidative stress. One of the most innovative approaches to enhance the therapeutic effects of bioactive compounds involves the use of liposomes, delivery systems that increase the bioavailability and stability of substances. Liposomes are spherical vesicles composed of one or more layers of phospholipids, capable of encapsulating compounds such as *P. heptaphyllum* extract, maximizing their effectiveness in combating oxidative and inflammatory damage. Obesity is often associated with oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant capacity. This stress plays a central role in the development of several comorbidities, such as dyslipidemia, insulin resistance, and cardiovascular diseases. Furthermore, the use of cyclophosphamide, an immunosuppressive agent, in experimental models can intensify oxidative and inflammatory effects, creating an ideal scenario to test the efficacy of natural substances with antioxidant and anti-inflammatory properties. In the studies analyzed, in addition to a literature review on *P. heptaphyllum*, experimental models were used that induced obesity in animals through a high-calorie diet, followed by treatment with the ethyl acetate fraction of the *P. heptaphyllum* leaf extract encapsulated in liposomes. Furthermore, the chemoprotective action of the ethyl acetate fraction of *P. heptaphyllum* extract, encapsulated in liposomes, against oxidative damage caused by cyclophosphamide was investigated, allowing a comprehensive evaluation of the protective effects of the treatment. The results of these studies indicate that the use of *P. heptaphyllum*, especially in the liposome-encapsulated form, may play a significant role in attenuating the effects of oxidative stress, inflammation and metabolic disorders associated with obesity and cyclophosphamide-induced stress. The combination of the antioxidant and anti-inflammatory actions of flavonoids, potentiated by liposomes, reveals a promising strategy in combating the negative effects of metabolic and immunosuppressive conditions.

Keywords: herbal treatment, flavonoid, oxidative stress.

SUMÁRIO

INTRODUÇÃO GERAL

Protium heptaphyllum (*P. heptaphyllum*), popularmente conhecida como breu-branco, é uma árvore nativa da Amazônia e de outras regiões tropicais da América do Sul. Tradicionalmente utilizado por suas propriedades medicinais, o breu-branco tem atraído a atenção da comunidade científica por seu vasto potencial terapêutico. Sua resina e folhas são ricas em compostos bioativos, como flavonoides, triterpenos (α e β-amirina), ácidos graxos e óleos essenciais, que demonstram atividades antioxidantes, anti-inflamatórias e antimicrobianas (Maia et al., 2000; Bandeira e Pessoa, 2002; Marques et al., 2010; Islam et al., 2014).

Uma abordagem inovadora no uso de *P. heptaphyllum* é a encapsulação de seus extratos em lipossomas. Esses sistemas de liberação controlada, compostos por camadas de fosfolipídios, oferecem maior estabilidade e biodisponibilidade aos compostos bioativos, potencializando seus efeitos terapêuticos (Gad et al., 2023; Anand et al., 2023). A encapsulação do extrato em lipossomas é particularmente promissora no tratamento de complicações associadas à obesidade, como resistência à insulina e doenças cardiovasculares, onde o estresse oxidativo desempenha um papel central (Kumar Vishwakarma et al., 2024; Chauhan et al., 2024).

O estresse oxidativo, caracterizado pelo desequilíbrio entre a produção de espécies reativas de oxigênio (EROs) e a capacidade do organismo em neutralizá-las, está associado a uma série de doenças crônicas, incluindo obesidade, diabetes, doenças cardiovasculares e câncer (Durairaj, 2014; David e Bender, 2021). A *P. heptaphyllum* se destaca no combate ao estresse oxidativo, graças aos seus compostos antioxidantes, sugerindo que o extrato pode ser uma valiosa terapia complementar para doenças inflamatórias crônicas (Rudiger e Valdir, 2013).

A obesidade, considerada um dos maiores desafios de saúde pública global, está associada a diversas comorbidades, como diabetes tipo 2, hipertensão e doenças cardiovasculares. Na fisiopatologia da obesidade, o estresse oxidativo e a inflamação desempenham papéis fundamentais. Estudos recentes (Carvalho et al., 2017), indicam que os triterpenos presentes em *P. heptaphyllum*, como α e β-amirina, podem modular vias metabólicas cruciais para essa condição, promovendo a redução de marcadores inflamatórios e a melhora da sensibilidade à insulina. Além disso, diversas pesquisas identificaram efeitos anti-inflamatórios (Holanda Pinto et al., 2008), antidepressivos (Aragão et al., 2006), antiobesidade

(Carvalho et al., 2015), gastroprotetora (Oliveira et al., 2004), antibacteriana (Cabral et al., 2018) e antioxidante (Patias et al., 2021), entre outras. A tecnologia de encapsulação em lipossomas, ao aumentar a biodisponibilidade desses compostos, amplifica seus efeitos terapêuticos, tornando-os ainda mais eficazes em contextos de obesidade induzida por dietas ricas em gorduras.

Outro campo promissor para o uso de *P. heptaphyllum* é a sua atividade antimutagênica e antimicrobiana. Estudos como o de De Lima et al. (2016), revelaram que o óleo essencial da resina possui significativa atividade antimutagênica, sugerindo seu potencial na prevenção de mutações genéticas que podem levar ao câncer. As propriedades antimicrobianas também foram destacadas, com ação contra bactérias patogênicas, atribuídas a monoterpenos como α-pineno e β-pineno (Cabral et al., 2020; De Lima et al., 2016).

Dentre tudo isso, nosso trabalho reúne quatro estudos fundamentais que trazem mais informações sobre a *P. heptaphyllum* e analisam o impacto de seus compostos bioativos em diferentes contextos experimentais. A seguir, será apresentada uma breve revisão desses artigos, que são anexados integralmente no trabalho.

O primeiro artigo, intitulado "Potential Antioxidants and Other Biological Activities of *Protium heptaphyllum* (Aubl.): Mini-Review" (Patias et al., 2023), oferece uma revisão abrangente sobre as propriedades antioxidantes e biológicas dessa planta. Este trabalho destaca os principais compostos bioativos, como α e β-amirinas e flavonoides, e seus mecanismos de ação. A revisão abrange os estudos mais relevantes sobre as atividades antioxidantes, anti-inflamatórias e antimicrobianas de *P. heptaphyllum*, demonstrando como esses compostos podem ser utilizados no combate ao estresse oxidativo e inflamações. Além disso, o artigo discute o uso da planta em diferentes condições experimentais, como diabetes, obesidade e doenças inflamatórias, fornecendo uma base teórica robusta para estudos posteriores sobre o uso terapêutico de *P. heptaphyllum*.

O segundo estudo, "Effect of Liposomal Extract of *Protium heptaphyllum* in the Treatment of Obesity Induced by a High-Calorie Diet" (Patias et al., 2024), investiga o impacto do extrato lipossomal de *P. heptaphyllum* no tratamento da obesidade em ratos submetidos a uma dieta hipercalórica. O estudo mostrou que a encapsulação dos compostos em lipossomas aumentou significativamente sua biodisponibilidade, resultando em uma maior eficácia no combate ao estresse oxidativo e à inflamação. Os resultados indicaram uma redução nos marcadores inflamatórios e uma melhora nos parâmetros metabólicos, como os níveis de glicose e lipídios. Este estudo é particularmente relevante por demonstrar como a tecnologia de lipossomas pode potencializar os efeitos terapêuticos de substâncias naturais no tratamento de

doenças metabólicas complexas como a obesidade.

O terceiro estudo, "Study of Liposomes Containing Extract from the Leaves of *Protium heptaphyllum* in Animals Submitted to a Mutagenic Model Induced by Cyclophosphamide" (Patias et al., 2024), explora os efeitos do extrato de *P. heptaphyllum* encapsulado em lipossomas em um modelo de mutagênese induzido por ciclofosfamida, um agente imunossupressor amplamente utilizado na quimioterapia. O estudo mostrou que o extrato encapsulado proporcionou uma proteção significativa contra os danos oxidativos causados pela ciclofosfamida. Além disso, os lipossomas permitiram uma liberação controlada e direcionada do extrato, aumentando sua eficácia no combate aos efeitos adversos da mutagênese induzida. Esses achados indicam que o uso de *P. heptaphyllum* encapsulado pode ser uma abordagem promissora na redução dos efeitos colaterais de agentes quimioterápicos.

O quarto artigo, "Effect of *Protium heptaphyllum* Extract on Metabolic Parameters in Obese Rats" (Patias et al., 2024), investigou os efeitos do extrato de *P. heptaphyllum* em parâmetros metabólicos de ratos obesos. O estudo demonstrou que o tratamento com o extrato resultou em melhorias significativas no controle de peso, nos níveis de glicose e nos perfis lipídicos dos animais. Além disso, o extrato reduziu os níveis de inflamação sistêmica, sugerindo seu potencial como tratamento complementar para obesidade e suas comorbidades, como diabetes e doenças cardiovasculares. Este estudo reforça o papel do *P. heptaphyllum* como um agente natural capaz de modular processos metabólicos e inflamatórios associados à obesidade.

Protium heptaphyllum emerge como uma planta de grande valor terapêutico, com propriedades antioxidantes, anti-inflamatórias, hipolipemiantes e antimutagênicas que podem ser aplicadas em diversas condições de saúde, como obesidade, diabetes, doenças cardiovasculares e até na prevenção de mutações genéticas. O uso de lipossomas para encapsulação de seus extratos bioativos amplia ainda mais seu potencial, melhorando a estabilidade e a biodisponibilidade dos compostos. Com base nos estudos recentes, o breu-branco continua a ser uma promessa valiosa na pesquisa de novos tratamentos naturais, especialmente para condições relacionadas ao estresse oxidativo e inflamação. O avanço no entendimento dos mecanismos de ação de seus constituintes bioativos, assim como o desenvolvimento de novas tecnologias para sua administração, como os lipossomas, abre caminhos promissores para o uso terapêutico dessa planta na medicina moderna.

CAPÍTULO 1

Antioxidants Potential and Other Biological Activities of *Protium heptaphyllum* (Aubl.): Mini-Review.

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MINI-REVIEW ARTICLE

Antioxidant Potentials and other Biological Activities of *Protium heptaphyllum* (Aubl.). March: Mini-Review

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Abstract: ***Background*:** Oxidative stress occurs when the cell's antioxidant defense system is insufficient. This can be corrected by active antioxidant substances, which help to eliminate the consequences of the damage caused or prevent the system from reaching the stress level.

***Objective*:** The actions of antioxidants can inhibit or delay tumor cells' appearance, delaying aging, and preventing other cellular damage resulting from the redox imbalance. Therefore, the present work aimed to research studies already published on *Protium heptaphyllum* (*P. heptaphyllum*) and its biological activities, mainly antioxidant effects once resulting from phenolic compounds, such as flavonoids, present in the plant.

***Methods*:** The methodology used was a literature review where information was collected from several studies related to *P. heptaphyllum*, oxidative stress, polyphenols, and flavonoids in databases, such as Scielo, PubMed, Google Scholar, LILACS, Chemical Abstract, ScienceDirect, among others in the period from 2002 to 2021.

***Results*:** The main studies carried out with metabolites of *P. heptaphyllum* demonstrated several biological activities such as antioxidant, gastroprotective, anti-inflammatory, analgesic, anxiolytic, antihyperglycemic, hyperlipidemic, among others. Although *P. heptaphyllum* has been little investigated by pharmacological studies, the results reported in this work may contribute to this plant species' chemical/pharmacological knowledge. Therefore, the secondary metabolites present in the plant may become test targets in future clinical trials for the drug arsenal.

***Conclusion*:** It can be observed that *P. heptaphyllum* is a promising source of secondary compounds, mainly flavonoids.

Keywords: Almacega, oxidative stress, flavonoids, polyphenols, antioxidants, *Protium heptaphyllum*.

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1. INTRODUCTION

Plants and plant extracts have great relevance in the treatment of various diseases. As a result, the interest of the population and the scientific community is increasing in the face of compounds that contain active metabolites such as polyphenols and triterpenes, which in most cases have antioxidant properties capable of combating oxidative stress [1].

These substances are related to beneficial effects on the body, contributing to a continuous improvement of health, mainly delaying aging and preventing various pathologies. Small molecules can exert an antioxidant effect, either acting directly on the neutralization of free radicals or indirectly participating in enzymatic systems with this function [2-5]. These molecules may have anticancer activity [3-7], anti-inflammatory, antimicrobial [5], vasorelaxant actions, reduce oxidative stress and blood pressure [8], and increase enzyme activity [9], among others.

Free radicals have been identified as the cause of oxidative stress that compromises the physiological processes of various organs and systems, especially neurodegenerative, chronic inflammatory, cardiovascular, and neoplastic diseases [10]. Oxidative stress needs to be better studied, but it is already well known that its importance has been growing given the findings already made. Considering the fight against this oxidative stress, polyphenols, especially flavonoids, stand out as antioxidants with great potential [11].

For this, an antioxidant is a molecule capable of inhibiting the oxidation of other molecules [12]; when present in low concentration to the oxidizable substrate, it regenerates the substrate or significantly prevents its oxidation. Antioxidants are molecules that prevent oxidation caused by free

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radicals and can interact with and stabilize free radicals. In this way, anti-oxidants are capable of reducing the rate of oxidation reactions of lipid compounds present in a given product. In addition, natural antioxidants are present in almost all plants, microorganisms and animal tissues. Many medicines are sourced from natural products or are inspired by natural products [12].

Polyphenols and flavonoids that are compounds present in plants, and have an ideal structure to scavenge free radicals. The antioxidant activity of flavonoids depends on their structure. It can be determined by five factors: reactivity as an electron donating agent, stability of the flavonol radical formed, reactivity against other antioxidants, ability to chelate transition metals, solubility, and interaction with membranes. Another important factor influencing the antioxidant activity of flavonoids is their interaction with biomembranes [13, 14].

Polyphenolic compounds such as flavonoids have been used in various drugs and food products because of their potential health benefits. Because of their antioxidant action, many phenolic compounds present in natural foods can reduce the risk of serious health problems. Flavonoids are ubiquitously found in plants as a member of polyphenolic molecules that share diverse chemical structures and properties. Flavonoids are cyclized diphenylpropanes that generally occur in plants and particularly in plant foods [15]. Many of these compounds, such as plant phenolics, often exhibit antioxidant activities; therefore, the addition of these compounds in food products can be useful for the health of consumers and also for the stabilization of food products. Due to the presence of some of these effective compounds, such as flavonoids, phenolic acids, and their esters in natural products (plants and their extracts), the positive physiological properties and the proven non-toxicity of these products, they can be used as antioxidants and mild preservatives [15].

There is growing evidence that the consumption of a variety of phenolic compounds present in natural foods can decrease the risk of serious health disorders due to their antioxidant activity, among other mechanisms [16]. *Protium heptaphyllum* (*P. heptaphyllum*) is a plant commonly known as “almacega”, “almiscar” or “breu branco”, typically found in the Amazon region. Its use in folk medicine is presented through the leaves and resin due to its stimulating, anti-inflammatory, and healing properties. As it is a plant rich in triterpenes and flavonoids, its resin has been the subject of several studies in recent years, which have shown many bioactive properties (Fig. **1**) [17-22].

This literature review was carried out using the terms “oxidative stress”, “free radicals”, “reactive oxygen species”, “antioxidants”, “polyphenols”, “Burseraceae” and “*Protium heptaphyllum*”, as well as their equivalents. The variety of terms used allowed a significant scope, to carry out a broad search on the topic. And it aimed to research the benefits of *P. heptaphyllum* and verify possible antioxidant effects from phenolic compounds, especially flavonoids, present in the plant. Considering the records found on the activity of secondary metabolites of *P. heptaphyllum*, it is believed that it is a promising source to be investigated and possibly become an active agent for pharmaceutical use. The methodology used was a literature review. Information was collected from several studies related to *P. heptaphyllum*, oxidative stress, polyphenols, and flavonoids in databases such as Scielo, PubMed, Google Scholar, LILACS, Chemical Abstract, ScienceDirect, and others in the period 2002 to 2021.

Fig. (1). *Protium heptaphyllum* Alb. Machand (**a**) Fruits and leaves [21]. (**b**) Trunk and resin [22]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2. METHODOLOGY

The methodology used was a literature review where information was collected from several studies related to “*Protium heptaphyllum*”, “Burseraceae”, oxidative stress, “free radicals”, “reactive oxygen species”, “polyphenols”, as well as their equivalents. The various terms used in databases such as Scielo, PubMed, Google Scholar, LILACS, Chemical Abstract, ScienceDirect, among others from 2002 to 2022. The online search in newspapers, articles, manuals, and books that described themes related to the aspects themes, with the following descriptors cited. The sample consisted of 89 (eighty-nine) scientific articles and 05 (five) books according to the aforementioned vehicles. As for the eligibility criteria, it is considered: Publication vehicle: scientific articles, manuals and textbooks; year of publication: from 2002 to 2022 and references whose study objective was a consequence of the theme. The results were discussed in context. And in this context, considering the records found on the activity of secondary metabolites of *P. heptaphyllum*, it is believed that it is a promising source to be investigated and possibly become an active agent for pharmaceutical use.

3. RESULTS

3.1. Oxidative Stress

Oxidative stress is the body's inability to defend itself from reactive oxygen and nitrogen species, causing structural and functional damage. So oxidative stress is a situation generated by an imbalance. It presents itself by the induction of more oxidative species being generated than the amount of anti-oxidative species being produced in defense [23]. All cellular compounds are subject to oxidative damage and the main causes of this damage are free radicals [24]. When derived from oxygen metabolism, these radicals are called Reactive Oxygen Species (ROS). This oxidative stress is referred to as one of the major causes of diseases such as cancer, diabetes, hypertension, and atherosclerosis [25]. The ROS and Reactive Nitrogen Species (RNS) can be highly reactive in the organism, attacking lipids, proteins, and

DNA. Others are reactive only in lipids and some are a little reactive, but despite that, they can generate harmful species. The main ROS and RNS are listed in Table **1** [24, 25].

Table 1. Main radical and non-radical ROS and ERN.

Main Radical ROS	Major Non-radical ROS	RNS
Hydroxyl ($HO^{\cdot}$)	Oxygen singlet ($^1O_2$)	Nitric oxide ($NO^{\cdot}$)
Superoxide ($O_2^{\cdot -}$)	Hydrogen peroxide (H_2O_2)	Nitrous oxide (N_2O)
Peroxyl ($ROO^{\cdot}$)	Hypochlorous acid ($HClO$)	Nitrous acid (HNO_2)
Alkoxyl ($RO^{\cdot}$)	-	Nitrites (NO_2^-)
-	-	Nitrates (NO_3^-)
-	-	Peroxynitrites ($ONOO^-$)

Stable atoms have an even number of electrons, which orbit around the nucleus in pairs. During this oxidation process, a stable atom loses an electron, gets a half-filled orbital, and becomes an unstable atom, called a free radical. Free radicals are molecules that lack stability because a half-filled orbital has an odd number of electrons [24]. Species of this type are very unstable and have high reactivity. In search of their stability, these molecules react against everything they find in front of them to usurp the electron they need, creating a chain reaction that will lead to cell destruction because removing an electron from a stable cell destabilizes the cell and the cell itself will become another free radical [26]. Several factors lead to the formation of free radicals, such as ultraviolet radiation, pollution, stress, smoke, food, few hours of sleep, *etc*. [27].

So, this condition generating oxidative stress is a harmful process that can negatively affect various cellular structures such as membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid [28], by acting at different levels of the cell, being the cytoplasm that triggers perturbations of the oxidation-reduction state. It also acts at the level of the cell nucleus (causing severe DNA damage) and on the membranes due to the composition rich in fatty acids, causing lipid peroxidation, which results in the alteration of its structure and functionality, modifying the fluidity, the potential and the transport of the membrane [27]. If not strictly controlled, oxidative stress can be responsible for induchronic and degenerative diseasesaccelerating the body's aging process and causing acute pathologies (such as trauma and stroke) [28]. Another factor that triggers or influences the oxidative stress process can be cited as energy-consuming sports activities that increase ROS production, such as high O_2 consumption [25].

Among other factors that lead to the formation of ROS, exposure to ultraviolet radiation (UV) A and B is one of the main ones. This radiation leads to the activation of metalloproteinases, collagenases, and elastases that destroy collagen, elastin, and other extracellular matrix proteins. From there, they also inhibit the formation of new fibers, both collagen and elastin [29]. UV radiation also affects the dermal micro vascularization of the dermis, causing dilation of the vessels with the deposition of amorphous substances composed of multiple laminations and material similar to the basement membrane and endothelial cells with organelles and pyknotic vesicles. Neoangiogenesis still occurs due to altered vessel structure [27]. Besides, stresses that cause damage to DNA and RNA are the most serious. If the DNA strand is broken, it can be connected at another position, thus altering the order of its bases. This is one of the basic mutation processes, and the accumulation of damaged bases can trigger oncogenesis. In addition, the alteration of amino acids in the primary sequence of enzyme disrupts its activity and when damage occurs in the cell membrane, it causes ruptures, as lipid oxidation generated interferes with active and passive transport normally across the membrane leading to cell death [11, 30].

The aging process or the exact mechanism of aging induced by oxidative stress is still unclear, but increased levels of ROS likely lead to cell senescence. This physiological mechanism prevents cell proliferation in response to the damage that occurs during replication. Senescent cells acquire a secretor phenotype associated with irreversible senescence involving the secretion of soluble factors (interleukins, chemokines, and growth factors), and degradative enzymes such as matrix metalloproteases (MMPs), and insoluble protein/extracellular matrix (ECM) components [31].

Aging is a loss of homeostasis due to chronic oxidative stress that mainly affects regulatory systems such as the nervous, endocrine, and immune systems. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in which chronic oxidative stress and inflammation feed each other and consequently increase age, morbidity, and mortality [32]. Thus, oxidative stress, cellular senescence, and other factors are involved in several acute and chronic pathological processes [33, 34].

As already explained, there are several factors for oxidative stress because both cells and organs are subjected in their normal aerobic metabolism to continuous exposure to oxidants. On the other hand, antioxidants can be generated through endogenous cellular sources as well as exogenous sources [31, 32].

This condition, called oxidative stress, occurs when the cell's antioxidant defense system is insufficient and can be partially or corrected by active antioxidant substances, which help to eliminate the consequences of the damage caused or prevent the system from reaching the stress level [27]. The equilibrium is restored by oxidation, which is the loss of this free electron, or by reduction, which is the gain of another electron. The oxidation phenomenon occurs in a chain where the molecule does not disappear; the electron can pass to other molecules successively [25].

According to Pizzino *et al*. [28], the human body has implemented several strategies to neutralfree radicals and oxidative stress effects, based on enzymatic antioxidants. Alongside these are several exogenous antioxidant molecules of animal or plant origin, introduced mainly through diet or nutritional supplementation. Among these various antioxi-

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dants, some have been explored in recent years for their real or supposed beneficial effect against oxidative stress, such as vitamin A, C and E, flavonoids, and polyphenols.

Antioxidants can be defined as natural or synthetic substances added to products to prevent or delay their deterioration by the action of oxygen. In medicinal biochemistry, antioxidants are defined as enzymes or other organic substances, such as vitamin C, E or β-carotene, capable of neutralizing the harmful effects of oxidation in animal tissues. Likewise, these compounds can directly neutralize the effects of free radicals or indirectly participate in enzymatic systems that have this function [35]. Antioxidant compounds are widely used in the production of cosmetics, medicines and foods, not only as preservatives but also as a fundamental raw material [36]. Finding antioxidant properties in plants and derived compounds requires appropriate methods to describe the mechanism of antioxidant activity and focus on reaction kinetics. Inhibited autoxidation is a suitable method for antioxidants that enhance termination and break the chain, while specific studies are needed for preventive antioxidants [37]. There is differentiation regarding the mechanism of combating ROS, and antioxidants can be classified as primary (acting as proton donors and preventing the initial process of action of free radicals) and secondary, which act in the decomposition of peroxides and hydroperoxides [38].

3.2. Phenolic Compounds

Phenolic acids are divided into benzoic acid derivatives and cinnamic acid derivatives. Phenolic acids are characterized by having an aromatic ring (benzene), a carboxylic group, and one or more hydroxyl and/or methoxyl groups in the molecule, conferring antioxidant properties to vegetables [39].

Cinnamic acid derivatives, generally in the form of phenylpropanoicheterosides, with a C6-C3 base structure, are the most widespread [40]. The phenolic compounds derived from benzoic acid have a C6-C1 backbone structure [39, 40]. Still, they contain wide structural variation with the presence of different substituents (hydroxyl or methoxyl) on one or more common aromatic skeletons. They can be classified according to their basic skeleton, where C6 corresponds to the aromatic ring plus branching with different carbon atoms depending on the class [39].

The classification of phenolic compounds taking into account only the chemical structure is not appropriate, as compounds containing phenolic hydroxyls may belong to other classes of metabolites. Then, they can also be classified into widely distributed phenolic compounds and restricted distribution phenolic compounds [39].

Animals cannot synthesize the aromatic ring, which is necessary to acquire them through the diet. Vegetables and microorganisms synthesize aromatic compounds and, from them, phenolic compounds [39]. Phenolic compounds constitute a very numerous and ubiquitous group of plant metabolites. They range from simple molecules to molecules with a high degree of polymerization. They are present in vegetables in free form or linked to sugars (glycosides) and proteins [41].

Among the natural antioxidants, phenolic compounds are abundant in the plant kingdom. They are found in leaves, flowers, seeds, fruits, bark, and wood and can accumulate in large amounts in plant organs or tissues. Phenolic compounds are considered secondary plant substances without involvement in metabolic pathways responsible for growth and development [42]. Organisms that synthesize these compounds do so primarily to attract pollinating and seed-dispersing animals and in response to stressful conditions, such as oxidative damage, attack by pathogens and insects, ultraviolet (UV) radiation, and injury [43].

Phenolic compounds are included in the category of free radical scavengers, being very efficient in preventing autoxidation [44]. Regarding their antioxidant action, synaptic, ferulic, and p-coumaric acids are more active than benzoic acid derivatives such as protocatechuic, syringic, and vanillic. This action is due to the double bond in the molecules of the cinnamic derivatives, which participate in the stability of the radical by unpaired electron displacement resonance, while the benzoic acid derivatives do not have this characteristic [45].

Studies presented in a review form demonstrated that polyphenols present in foods such as cocoa, coffee, teas, fruits, grapes (and derivatives), and olives (and derivatives) have the potential to play a beneficial role in endothelial function [46]. Despite this, it emphasizes the multiple varieties of polyphenols present in nature and the issue of bioavailability that can alter their absorption.

Among the various classes of existing polyphenols are flavonoids with relevant antioxidant capacities. These molecules are abundantly present in secondary metabolites of the plant kingdom and almost non-existent in algae and fungi. Its structural form is diversified, mainly represented by 15 carbon atoms in its fundamental nucleus, consisting of two phenyls linked by a chain of three carbons [39]. Its structure then consists of two aromatic rings (A and B), joined by three carbons that form a heterocyclic ring (C) (Fig. **2A**). Ring A is derived from the acetate/malonate cycle, while ring B is derived from phenylalanine. The C ring can vary and result in important classes of flavonoids such as flavones, flavanones, flavonols, anthocyanins, and isoflavones. Substitutions in the A and B rings can give rise to different compounds within each class of flavonoids, namely oxygenation, alkylation, glycosylation, acylation, and sulfation [41].

Flavonoids are natural compounds widely distributed in higher plants, mainly angiosperms, with about 5000 compounds described [47]. They are synthesized in plants and participate in the light phase of photosynthesis, during which they catalyze the transport of electrons. A large portion of them have bioactivities, such as antioxidant, anti-inflammatory, antibacterial, and tanning activities [48, 49].

Most flavonoids are extensively metabolized by the gut microbiota and host tissues. Flavonoid metabolism exhibits wide variation among individuals. Differences in flavonoid metabolism impact the immune system [50].

To exert antioxidant activity, flavonoids depend on their structure. This is best exemplified by 5 factors: reactivity as an H and electron-donating agent, stability of the formed

flavanoyl radical, reactivity against other antioxidants, ability to chelate transition metals, and solubility, and interaction with membranes. The oxidation potential of flavonoids is directly linked to the sequestering activity and the species to be sequestered [11] (Fig. **2B**).

The lower the flavonoid oxidation potential, the greater its activity as a free radical scavenger so flavonoids with oxidation potential lower than that of Fe^{+3} and Cu^{+2} and their complexes can reduce these metals, being potentially pro-oxidants, besides, the greater the number of hydroxyls, the greater the activity as an H and electron-donating agent. Monohydroxylated flavonoids show very low activity; for example, the 5-hydroxy flavone has activity below detection limits. The need for at least two phenolic hydroxyl groups in the flavonoid was found, demonstrating that monohydroxy flavonoids are ineffective. Another factor influencing flavonoid's antioxidant activity is their interaction with biomembranes [11].

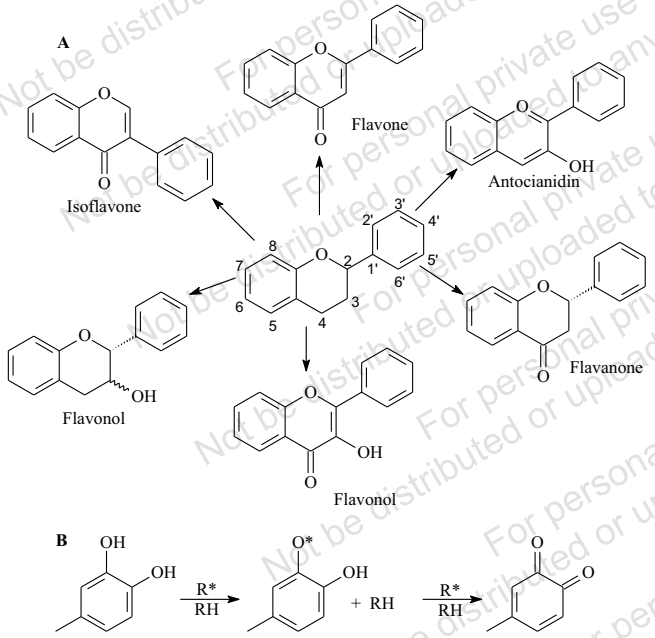


Fig. (2). (**A**) Fundamental chemical structure of flavonoids. (**B**) Flavonoids - free radical scavenging mechanism [93].

Studies show that flavonoids can reduce cellular stress. Cellular stress includes neuroinflammation, oxidative stress, proteotoxicity, and endoplasmic reticulum stress. Some diseases originating from cellular stress include Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. From this, the use of flavonoids, such as antioxidants, anti-inflammatory and anti-apoptotic, still needs to be better elucidated, as well as the cellular stress response that would prevent the pathogenesis of neurodegenerative diseases [51].

Flavonoids can inhibit viruses through several mechanisms. They can block the binding and entry of viruses into cells; interfere with various stages of viral replication processes or translation and processing of polyproteins to prevent the release of the virus from infecting other cells. Based on antiviral mechanisms of action, flavonoids can be prophylactic inhibitors, therapeutic inhibitors, or indirect inhibitors by interaction with the immune system [52]. Studies have shown that flavonoids may be a target to be tested in future clinical trials for the arsenal of drugs against Coronavirus infections. This is because secondary metabolites widely present in plant tissues with antioxidant and antimicrobial functions, in addition to inhibiting key proteins involved in the infectious cycle of the virus, may contribute to this [53].

3.3. *Protium heptaphyllum*

Scientific research in taxonomic, photochemical, and bioprospecting areas has been increasingly promising. This leads us to recover the importance of knowing nature itself and the resources used [54]. Bioprospecting for secondary metabolites is a key predictor of a country's economic growth and development. Regarding the biotechnological applications of research with bioprospecting activities in Brazil, it was observed that the pharmaceutical industry was responsible, from 2004 to 2018, for most investigations (72.9%), followed by agriculture (12.8%), the bioenergy industry (5.7%), bioremediation (4.3%) and food industry (4.3%) [55]. The data indicate a promising scenario for bioprospecting in the country. However, insects, sponges, and amphibians had a low number of published works, making a temporal analysis impossible [55].

Choosing the plant to be investigated is very important since this can be a source of active principles for treating diseases and can even be used in existing therapeutic combinations to enhance the pharmacological effect and reduce toxicity. Due to the vast chemical diversity and potential therapeutic action often found in plants, many researchers are focusing their investigations on the biological activity of natural products, even though these compounds do not directly serve as new drugs, but can provide information for the development of an important therapeutic agent [56].

It is already known to all those natural compounds are a promising source of components with biological activity. This can be proven through the work by Neuman *et al*. [57], who analyzed a large number of natural products used for new drugs over 4 decades. These same authors also report in their work the influence of Brazilian biodiversity on the discovery of these new drugs.

New research is always under development and this is verified through several studies. Among these, we can mention Rodrigues *et al*. [58], who demonstrated with their research the *in vivo* and *in vitro* ethnopharmacological use of *S. impressifolia* as a contributor to the development of new herbal medicines for the treatment of cancer. Brito *et al*. [59], observed that quercetin had an anti-proliferative effect on hepatocellular carcinoma cell lines. Pereira *et al*. [60], verified a reduction in tumor growth and the induction of cell death through treatment with *Olea europaea* L., in their investigations on the induction of cell death by apoptosis of lung cells (H4600). Mousa *et al*. [61], observed a great gastroprotective effect on ethanol-induced gastric ulcers in extracts of *Cuphea ignea*, and its effect may be mediated by suppression of oxidative stress and gastric inflammation. Cappello *et al*. [62], demonstrated the effects of *Citrus ber-*

gamia Risso flavonoids with a significant degree of hypocholesterolemic and antioxidant/free radical scavenging activities.

Many substances of plant origin are favorable sources for the development of new drugs. This scientific interest in new medicinal agents obtained from natural sources, especially plants, is due to the constitution of numerous molecules from their secondary metabolism [63]. Many medicines are derived directly or indirectly from plants and contain several phytoconstituents with unique and distinct properties [20].

Protium heptaphyllum (Aubl.) March. (*P. heptaphyllum*), is a plant commonly known as "almacega", "almíscar" or "white pitch", found in abundance in South America in various parts of Brazil and typically in the Amazon [64]. Its use in folk medicine is presented through the leaves and resin due to its stimulating, anti-inflammatory, and healing properties. The Burseraceae family comprises18 genera and over 700 species [14].

The Burseraceae family is native to North America, and due to migrations, it has moved to tropical and subtropical regions around the world. It is classified in the order Sapindales, class Dicotiledoneae, and subclass Rosidae. In neotropical regions, it is mainly represented by 228 species comprising 8 genera that can be classified into three tribes: Burseraceae, Canarieae, and Protieae. The genus *Protium* (Tribe Protieae) is the main member of the family with 150 species [14, 64].

Protium is the most heterogeneous genus of the family and the main representative in South America, then divided into 135 known species. The identification of *Protium* trees is not easy because, during non-flowering periods, they are confused with other species of Burseraceae. Another impediment is the presence of trunk resins, buttresses (a species of tube roots), and compound leaves, which makes it confusing with the species of Anacardiaceae [14].

P. heptaphyllum is a tree that is around 10 meters high, with a trunk of 50 to 60 cm in diameter at the base, with dark red bark and opposite leaves, small flowers in terminal panicles, and its fruits are reddish drupes, containing one to four seeds [65]. It presents good quality wood for joinery, carpentry, and firewood [66].

Species of the Burseraceae family are described in the literature as they present compounds with anti-inflammatory [67], and antimicrobial and antioxidant activity [68]. In folk medicine, this resin is used for various purposes due to its stimulating, anti-inflammatory, and healing properties and as incense in ritual religions, it also has aromatic characteristics [64].

Resins of the genus *Protium* are extremely rich in terpenes and flavonoids, mainly pentacyclic triterpenes, molecules formed by at least 2 isoprene units, following the chemical formula $(C_5H_8)n$, being divided into subclasses according to the number of isoprene units [63-69]. As for the essential oils obtained from the resin are mostly made up of monoterpenes [19].

The plant, rich in volatile monoterpenes, exhibits a chemical composition that can be strongly altered over time. Based on this, Albino *et al*. [70], made considerations in their work on the temporal changes of the volatile composition of these oleoresins, noting that some triterpenes were oxidized to p-cymene, which, in turn, was oxidized to p-cymene-8-ol during the natural aging of the exudate due to abiotic factors.

In addition, several other authors observed different compositions in the resin, leaves, flowers, and fruits of *P. heptaphyllum* as reported in Table **2**.

In a study to identify the volatile components of the leaves, flowers, resin of the stem, and bark of the branches of the tree *P. heptaphyllum* collected in the eastern Colombian plains, the composition found in the resin of the plant presented the characteristic pattern of the resins *Protium spp*.. The main constituents identified by GC-MS in the volatile fraction of the leaves were: guaiol, *α*-copaene, 1,10-di-epi-cubenol, trans-caryophyllene, and cadinene. Germacrene D, germacrene B, bicyclogermacrene, and limonene were identified in the flowers. While in the resin, p-cymene, *α*-pinene and limonene were identified. In the bark, germacrene D, 1,10-di-epi-cubenol, guaiol, and cadinene were found. So, the resin showed a high percentage of monoterpenes, the leaves showed a high proportion of oxygenated sesquiterpenes, and the flowers and bark showed a high composition of sesquiterpenes [71].

Because it is a plant rich in triterpenes and flavonoids, in recent years, it has been the subject of several types of research, which exhibited many bioactive properties such as anti-inflammatory [72], antidepressant [73], antiobesity [74], gastroprotective [75], antibacterial [76], antioxidant [77], among others. These properties will be better exemplified below.

Studies obtain evidence that *α* and *β*-amyrin triterpenes present in *P. heptaphyllum* delay acute inflammation in the rat model of periodontitis. Periodontitis is an inflammatory disease characterized by the accumulation of plaque that can initiate a local inflammatory response [74]. In addition, Oliveira *et al*. [75] observed the anti-inflammatory action of *P. heptaphyllum* through the model for four different aspects of the inflammatory process characterized by acute edema and increased vascular permeability induced by intraperitoneal injection of acetic acid and granuloma induced by pellets of cotton. The analgesic and anti-inflammatory activities of the isomeric mixture of *α* and *β*-amyrin from *P. heptaphyllum* were also confirmed by writhing and formalin tests in mice. The tests demonstrated peripheral and central analgesic effects independent of the opioid system in addition to potent anti-inflammatory activity. Anti-inflammatory activity was potentiated by both indomethacin and thalidomide, suggesting a potential involvement of prostaglandin and TNF-*α* inhibition [78].

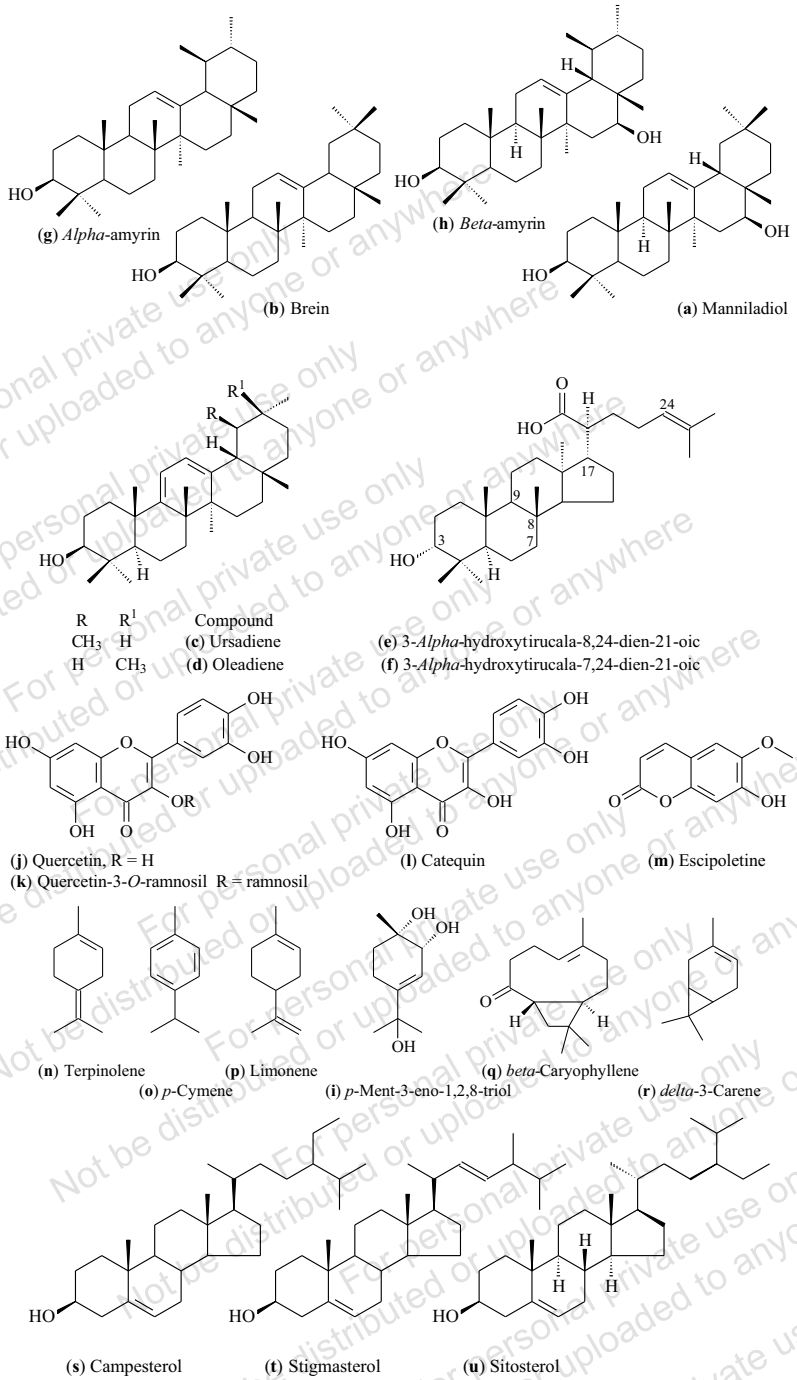
Studies that examined the anxiolytic and antidepressant effects of the *α* and *β*-amyrin mixture of triterpenes isolated from the stem bark resin of *P. heptaphyllum* demonstrated action at benzodiazepine-like receptors and also an antidepressant effect where noradrenergic mechanisms likely play a role [73]. It was also observed by Aragão *et al*. [79] an anticonvulsant effect of the isomeric mixture of *α*- and *β*-amyrin from *P. heptaphyllum*. This anticonvulsant activity of *α*- and *β*-amyrin triterpenes are related to the GABAergic

Table 2. Main studies of *P. heptaphyllum* specifying the material used, the technique used, the main isolated metabolites and their structure. The structures of the main isolated metabolites are listed at the end of the table in alphabetical order (from a to u) as described above in the same table in the isolated metabolites column.

Material	Isolated Metabolites	Method	References
Oil-resin	Eight triterpenes, manniladiol (a), brein (b), ursa-9(11):12-dien-3beta-ol (c), olean-9(11):12-dien-3beta-ol (d), 3alpha-hydroxy-tirucalla-8,24-dien-21-oic acid (e), 3alpha-hydroxy-tirucalla-7,24-dien-21-oic acid (f), alpha-amyrin(g) and beta-amyrin (h)	^{13}C NMR data and mass spectra	[17]
Stems, including peels, and leaves	Cumarins, cumarinolignoides, and terpenes	Chromatographed on a silica gel column and NMR spectra	[69]
Resin, fruit, leaves, and trunk	Monotherpene insulation p-menth-3-ENE-1,2,8-triol (i), alpha-amyrin (g) *beta*-amyrin (h), quercetin (j), brein (b), quercetin-3-O-ramnosil (k), (-) catechin (l), and escipoletine (m)	Structures were established by 1D and 2D RMN Spectroscopy and comparison with published data	[18]
Resin	A binary blend of alpha-amyrin (g) and *beta*-amyrin (h)	The compounds obtained were characterized by spectroscopic data such as IR, 1 h, and ^{13}C NMR and comparison with the literature	[68]
Fruits and leaves	Limonene (p) (92.68%), δ-3-carene (r) (63.86%) does not contain fruit essential oil. *β*-caryophyllene (q) (32.08% in the hydrodistillation and 29.07% in the had space), constituents of the leaves	Hydrodistillationtechnique in modified Clevenger device and dynamic headspace with porapak-q	[65]
Volatile constituents from resin	Terpinolene (n) (42.31%) and *p*-cymene (o) (39.93%)	Extracted by hydrodistillation with an 11.3% (PHH)	[19]
Stem	Alpha-amyrin (g) and *β*-amyrin (h) terpenoids, the steroids campesterol (s), stigmasterol (t), and sitosterol (u), and the coumarinscopoletin (m)	Column chromatography (CC), RMN H1 and C13 spectra, UV and IV	[67]
Volatile composition Oleo resins	High terpinolene (28.2-69.7%), while the elderly contained large amounts of p-cymene (o) (18.7-43.0%) and p-Cymen-8-OL (8.2-31.8%)	Multivariate analyzes were performed based on yield and the main essential components of the oil to demonstrate the existence of two subsets. Analysis of partial genome sequencing of the species, producing the largest amount of data for the gender of Protium. Subsequently, they were searched by nucleotide sequences responsible for the enzymes involved in the biosynthesis of monoterpenes	[70]
Volatile fraction of leaves	Leaves: guaiol (14.4%), alpha-copaene (8.6%), 1,10-di-epi-cubenol (8.1%), beta-caryophyllene (5.7%) and gamma-cadinene (5.4%). Germacrene D (13.9%), germacrene B (13.4%), bicyclogermacrene (11.8%) and limonene (p) (8.3%) flowers, while *p*-cymene (o) (30.1%), alpha-pinene (22.1%) and limonene (p) (14.4%) were identified in the resin; finally, germacrene D (27.7%), 1,10-di-epi-cubenol (7.9%), guaiol (7.4%) and gamma-cadinene (6.9%) were found in the bark	GC-MS	[71]

(Table 2) Contd….

Isolated Metabolites



system. They may be linked to the inhibition of the protein kinase C (PKC) signaling cascade and also to changes in amino acid metabolism.

Investigations of the anti-obesity properties of *P. heptaphyllum* resin and its possible mechanisms in mice fed with a high-fat diet were determined. This study suggested that the anti-obesity potential of *P. heptaphyllum* is largely due to its modulatory effects on various hormone and enzyme secretions related to fat and carbohydrate metabolism and the regulation of obesity-associated inflammation [64]. In another study, Carvalho *et al*. [80], also noted that triterpenes from *P. heptaphyllum*, *α*, *β*-amyrin may be beneficial in reducing high-fat diet-induced obesity and associated diseases *via* modulation of enzymes, hormones, and inflammatory responses.

Besides, studies have shown the anti-hyperglycemic and lipid-lowering effects of *α*, *β*-amyrin, in mice once these compounds significantly reduced the increases in blood glucose, total cholesterol, and serum triglycerides [81]. Furthermore, *α, β*-amyrin effectively reduced elevated plasma

Table 3. Main studies of *P. heptaphyllum* specifying the material used, the main metabolite tested, method and dose used, and main results.

Plant Part	Classification	Method	Dose	Result	References
Trunk Wood resin	Pentacyclic triterpenoids, *α*- and *β*-amyrin (45.25%), brein and maniladol (9.5%) and a small quantity of lupeone. Besides, it yielded an essential oil content of 0.7%	Male *Wistar* rats (150-200 g) and male Swiss mice (20-25 g)	200 and 400 mg/kg	Potential gastroprotective and anti-inflammatory properties of *P. heptaphyllum* resin also support its popular use in gastrointestinal disorders	[75]
Trunk Wood Resin	Triterpene mixture, *α*- and *β*-amyrin	Adult male *Wistar* rats (150-200 g)	50 and 100 mg/kg	Feasibility of developing herbal medicines for the treatment of liver disorders	[91]
Trunk Wood Resin	Triterpene mixture, *α*- and *β*-amyrin	Male *Swiss* mice 20-25 g	3, 10, 30 and 100 mg/kg	Triterpene, α-*β* amyrin has an analgesia-inducing effect, possibly involving the vanilloid receptor (TRPV1) and an opioid mechanism	[63]
Trunk Wood Resin	450 mg *α* and *β*-amyrin	Male *Swiss* mice (20-30 g)	10, 25 and 50 mg/kg	Sedative and anxiolytic effects that may involve action on benzodiazepine receptors, and also an antidepressant effect where noradrenergic mechanisms will probably have a role	[73]
Essential Oils from Leaves and Fruits	Fruits *α*-terpinene (47.6%). Leaf mainly contained sesquiterpenes as 9-EPI-cariofileno (21.4%), trans-isolegifolanone (10.7%) and 14-hydroxy-9-epi-cariophylene (16.7%)	Two-spotted spider mite *(Tetranychus urticae)*	5, 10, 15, 20 and 25 FL of essential oil corresponding to 2, 4, 6, 8 and 10 fl. l-1 air, respectively. Oil exposure period It was 24, 48 and 72 h	Mortality and detriment properties of oviposition in (10 fl. l-1 air), but only the essential oil of fruits induced *T. urticae* repellency	[92]
Trunk Wood Resin	450 mg *α* (67%) and *β* (33%) amyrin	Male *Swiss* mice (20-30 g) or male rats (200-250 g)	10, 50 and 100 mg/kg	Peripheral analgesic effects and independent plants of the opioid system and also showed a powerful anti-inflammatory activity	[78]
Trunk Wood Resin	Triterpene *α* and *β*-amyrin	Male Wistar rats weighing 160-200 g	5 and 10 mg/kg	Delay acute inflammation in a periodontitis mouse model and justify more studies on their effectiveness to prevent bone loss associated with chronic periodontitis	[72]
Trunk Wood Resin	*α* and *β*-amyrin , pentacyclic triterpenoid	Male *Swiss* mice 20-25 g	10, 30 and 100 mg/kg	Improves induction of cerulein acute pancreatitis acting as an anti-inflammatory and antioxidant agent	[82]
Essential Oil	The essential oil was purchased from Laszlo Aromatherapia Ltda. and was obtained from leaves and derived from this specimen by steam distillation	Male *Wistar* rats 180-250 g	12, 5, 25, 50 and 100 mg/kg	The essential oil exercises its gastroprotective activity, possibly increasing COX-2 and EGF expression due to its possible antioxidant property	[83]
Trunk Wood Resin	*α* and *β*-amyrin, a mixture of pentacyclic triterpene	Male *Swiss* mice weighing 25-30 g	10, 30, and 100 mg/kg	Antihyperglycemic and hypolipidemic effects of *α, β*-amyrin mix suggest that it could be a leading compound for the development of effective drugs in diabetes and atherosclerosis	[81]

(Table 3) Contd….

Plant Part	Classification	Method	Dose	Result	References
Trunk Wood Resin	α and β-amyrin (45.25%), Broine and Maniladiol (9.5%), and a small amount (1.25%) of a mixture of Lupenone and α and β-amirenone	*Swiss* Mice weighing 25-30 g	10 mg/kg and 20mg/kg	Antiobesity effect of *P. heptaphyllum* is largely due to its modulating effects on various hormones and enzymatic secretions related to the metabolism of fats and carbohydrates and the regulation of inflammation associated with obesity	[74]
Trunk Wood Resin	α (67%) and β-amyrin (33%)	Male *Swiss* mice 20-30 g	5, 10, 25, and 50 mg/kg	Amy's anticonvulsant activity is related to the gaberic system and may be linked to the inhibition of the PKC signaling cascade, as well as changes in amino acids metabolism	[79]
Oil Resin	Monotherpines, α-terpinolene (32.7%), limonene (22.0%) and 3-careno (15.0%)	Rats (*Rattus norvegicus*)	0.5% oil resin in isotonic solution	Beneficial in the cicatrization process of experimentally induced cutaneous wounds in rats, making it a therapeutic option in veterinary medicine	[89]
Resinous Exudate from the Trunk Wood	α and β-amyrin	Male *Swiss* mice weighing 20-25 g	10 or 20 mg/kg	Benefit in the reduction of obesity induced by fat diet and associated disorders *via* modulation of enzymes, hormones, and inflammatory responses	[80]
Essential Oil	Monoterpenes, being the terpinolene, *p*-cimene-8-ol, and *p*-cimene	-	25, 50 and 100 mg/kg	Essential oil presented antimut agenic activity due to its chemical composition	[88]
Resin Essential Oil	Limonene, α-terpineol, *p*-cineole, *o*-cymene and α-phelandrene	*Candida* spp.	Concentrations of 1000 µg/L, 500 µg/L, and 250 µg/L, protocol M44-A2 (CLSI 2009)	Considerable antifungal activity, which merits further investigation for alternative clinical applications	[86]
Trunk Wood Resin	Limonene, *p*-cineole, and *o*-cymene	Male *Wistar* normotensive rats (200-300 g)	3-750 µg/mL	In intact pre-contracted rings with phenylephrine (Phe 1 µm), EOPH (3-750 µg/mL) induced relaxation, and essential oil had a concentration-dependent vasorelaxant effect, without the involvement of endothelial mediators	[90]
Essential Oils of the Leaves of Ripe and Green Fruits	Myrcene (59.0%), β-element (17.2%), limonene (12.9%), spathulenol (12.6%), α-cubebene (11.6%), Germacrene D (10.6%), trans-nerolidol (9.8%) and α-cadinol (8.8%)	Minimum inhibitory concentrations of antibacterial *in vitro*	-	The essential oils of the ripe and green fruits presented the largest antibacterial activity against the anaerobic bacteria *Prevotella nigrescens* (CIM = 50 µg/mL). The foliar essential oil presented very promising activity against *Streptococcus mutans* (CIM = 50 µg/ml) and *Streptococcus mitis* (CIM = 62.5 µg/mL). The antibacterial activity of OES against oral pathogens is also described for the first time	[76]
Essential Oil from the Resin	22 constituents and β-phelandrene (60.68%) were the majority followed by p-cymene (13.63), α-pinene (4,47), and α-phelandrene (3.38%)	Halo inhibition. Inhibition of biofilm biomass and biomass reduction of biofilm	MIC and MBC were 11 mm, 2 mg/mL and 8 mg/mL, respectively. Biofilm biomass inhibition and preformed biofilm biomass reduction were detected at 4 mg/mL EOPH concentration	Release tests of cellular constituents and membrane permeability indicated that the plant can break the cell membrane, leading to extravasation of intracellular constituents such as reducing sugars and materials with an absorbance of 260 nm	[85]

(Table 3) Contd….

Plant Part	Classification	Method	Dose	Result	References
Essential Oil from the Resin	*p*-cymene (27.70%) andpinene (22.31%)	Third instar *A. aegypti* larvae	Nanoemulsion prepared at a concentration of 25,000 gmL^{-1} was diluted at concentrations of 2, 5, 10, 15, and 20 gmL^{-1} with a final volume of 100 mL solution to receive 10 larvae in each baquer	The nanobiotechnology product derived from the essential oil of the resin of *P. heptaphyllum* can be used against infectious vectors of disease	[87]
Ethyl Acetate Extract from Leaves	Quercetin-3-*β*-D-glycoside myricetin and quercetin	Male *Wistar* rats 28-38 g	100 mg/kg	This antioxidant effect may be due to the presence of the flavonoids identified, which are already well-known for their antioxidant properties	[77]
Trunk Wood Resin	13 compounds belonging mainly to ursano, oleanano, and tirucalan groups	THLE-3 cells ATCC® CRL-11233	-	A useful alternative to contrast hypercholesterolemia, highlighting its potential as a sustainable multilevel natural extract for the nutraceutical industry that is rapidly gaining acceptance as a source of compounds that promote health	[84]

glucose levels during the oral glucose tolerance test [81]. In addition, plasma insulin levels and histopathological analysis of the pancreas revealed the beneficial effect of *α*, *β*-amyrin in preserving beta cell integrity [82], suggesting that *α, β*-amyrin from *P. heptaphyllum* exerts an anti-adipogenic effect, mainly through the modulation of lipid and carbohydrate metabolism in 3T3-L1 cells. Therefore, the *in vitro* results suggest that *α*, *β*-amyrin can be developed as a new therapeutic agent for the treatment and prevention of obesity [83].

The gastroprotective activity was observed by Oliveira *et al*. [75], through tests of gastric damage induced by acidified ethanol, gastric secretion after four hours of suturing the pylorus, and investigation of non-protein sulfhydryl in gastric tissue. Araújo *et al*. [83], studying the essential oil of the plant, also observed gastroprotective activity, possibly by increasing the expression of COX-2 (Cyclooxygenase-2) and EGF (epidermal growth factor) and by its potential antioxidant property. In this study, the authors observed that the essential oil of *P. heptaphyllum* significantly inhibited the formation of ulcers induced by three different models, increasing the levels of GSH (reduced glutathione) and maintaining the same levels of SOD (superoxide dismutase) and GPx (glutathione peroxidase) in the treated group, inhibited MPO (myeloperoxidase) and MDA (lipid peroxidation), did not produce a significant change in gastric juice content.

More recently, Mannino *et al*. [84], studied the potential of *P. heptaphyllum* to reduce cholesterol. They identified tetra and pentacyclic triterpenoid acids as the main compounds responsible for the hypocholesterolemic action, highlighting the plant as potential as a sustainable natural extract for the rapidly growing nutraceutical industry gaining acceptance as a source of health-promoting compounds.

Studies with *P. heptaphyllum* essential oils using ripe and unripe fruits show greater antibacterial activity against anaerobic bacteria. The leaf essential oil showed very promising activity against *Streptococcus mutans* and *Streptococcus mitis* [76]. The larvicidal effect has also been detected. These authors carried out a work where they studied the chemical composition, antimicrobial activity, and antibiofilm of the bacterium *V. parahaemolyticus* in the essential oil *P. heptaphyllum*. Its mechanism of action seems to be related to the increase in cell membrane permeability [85, 86].

In line with this view, it was observed antifungal activity of *P. heptaphyllum* was against *Candida* species, using the disk diffusion method. The authors emphasize that the plant deserves further investigation for alternative clinical applications since this species is widely distributed and has good yields and important therapeutic applications [86].

The larvicidal effects were observed through the use of a nanoemulsion containing *P. heptaphyllum* essential oil with a residual effect of 72 hours after application. In this study, it was possible to conclude that the nanobiotechnological product derived from the oil of the plant in question can be used against vectors of infectious diseases [87]. In addition, another study evaluated the chemical composition of *P. heptaphyllum* essential oil and its cytotoxicity on a breast cancer cell line (MCF-7), antimicrobial activity, and antimutagenicity *in vivo*. The possible chemopreventive activity of the essential oil of *P. heptaphyllum* resin was observed and most likely due to the action of monoterpenes, it was possible to identify the antimutagenic effects and absence of cytotoxic and pro-apoptotic effects [88].

Regarding the topical use, Bernadi *et al*. [89], evaluated the clinical and histopathological aspects of the topical application of *P. heptaphyllum* resin oil in the healing process of cutaneous wounds induced experimentally in rats. Their investigation showed that the resin oil was beneficial in the healing process of skin wounds induced experimentally in rats, making it a therapeutic option in veterinary medicine.

Patias *et al*. [77], were the first to test the extract of *P. heptaphyllum* leaves against oxidative stress induced by ac-

etaminophen. In their studies, the authors observed a considerable amount of phenols and flavonoids and a high DPPH radical scavenging property. A fraction of the extract showed antioxidant activity to repair oxidative stress and, the extract showed a hepatoprotective effect and demonstrated a possible hypoglycemic effect of the plant. The authors believe that the antioxidant effects may be due to the presence of flavonoids identified by *in vitro* tests such as Quercetin-3-*β*-D-glucosylated, Myricetin, and Quercetin, which are already well known for their antioxidant properties [90-93]. Bandeira *et al*. [68], also observed *in vitro* antioxidant activity of the essential oil of *P. heptaphyllum* (Table **3**).

CONCLUSION

Several studies are related to *P. heptaphyllum*, but these are directed mainly to the presence of *α, β*-amyrin triterpenes in the resin from the plant stem. However, more studies should still be carried out with *P. heptaphyllum* to define better the participation of the plant in the face of oxidative damage. A further study with a longer period will be useful to verify, quantify and exemplify the mechanisms of action of other compounds present in the plant. Actively studying the flavonoids present in the leaves or fruits of this plant can bring results regarding the antioxidant potential. From this, the relevance of studies in the field of phytotherapy can be observed, as we have a rich biodiversity with great potential for the development of natural products which can act in the treatment of diseases.

LIST OF ABBREVIATIONS

ECM	=	Extracellular Matrix
MMPs	=	Matrix Metalloproteases
PKC	=	Protein Kinase C
RNS	=	Reactive Nitrogen Species
ROS	=	Reactive Oxygen Species
UV	=	Ultraviolet Radiation

CONSENT FOR PUBLICATION

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The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Oliveira, E.; Menini Neto, L. Ethnobotanical survey of medicinal plants used by residents of the village of Manejo, Lima Duarte-MG. *Rev. Bras. Pl. Med.* **2012**, *14*(2), 311-320.

[2] Ferrazzano, G.; Amato, I.; Ingenito, A.; Zarrelli, A.; Pinto, G.; Pollio, A. Plant polyphenols and their anti-cariogenic properties: A review. *Molecules,* **2011**, *16*(2), 1486-1507. http://dx.doi.org/10.3390/molecules16021486 PMID: 21317840

[3] Nageen, B.; Sarfraz, I.; Rasul, A.; Hussain, G.; Rukhsar, F.; Irshad, S.; Riaz, A.; Selamoglu, Z.; Ali, M. Eupatilin: A natural pharmacologically active flavone compound with its wide range applications. *J. Asian Nat. Prod. Res.,* **2020**, *22*(1), 1-16. http://dx.doi.org/10.1080/10286020.2018.1492565 PMID: 33511872

[4] Jiang, D.; Rasul, A.; Batool, R.; Sarfraz, I.; Hussain, G.; Mateen Tahir, M.; Qin, T.; Selamoglu, Z.; Ali, M.; Li, J.; Li, X. Potential anticancer properties and mechanisms of action of formononetin. *BioMed Res. Int.,* **2019**, *2019*, 1-11. http://dx.doi.org/10.1155/2019/5854315 PMID: 31467899

[5] Salehi, B.; Selamoglu, Z.; Sener, B.; Kilic, M.; Kumar Jugran, A.; de Tommasi, N.; Sinisgalli, C.; Milella, L.; Rajkovic, J.; Flaviana B Morais-Braga, M.; F Bezerra, C.; E Rocha, J.; D M Coutinho, H.; Oluwaseun Ademiluyi, A.; Khan Shinwari, Z.; Ahmad Jan, S.; Erol, E.; Ali, Z.; Adrian Ostrander, E.; Sharifi-Rad, J.; de la Luz Cádiz-Gurrea, M.; Taheri, Y.; Martorell, M.; Segura-Carretero, A.; Cho, W.C. *Berberis*. Plants-drifting from farm to food applications, phytotherapy, and phytopharmacology. *Foods,* **2019**, *8*(10), 522. http://dx.doi.org/10.3390/foods8100522 PMID: 31652576

[6] Nawaz, J.; Rasul, A.; Shah, M.A.; Hussain, G.; Riaz, A.; Sarfraz, I.; Zafar, S.; Adnan, M.; Khan, A.H.; Selamoglu, Z. Cardamonin: A new player to fight cancer *via* multiple cancer signaling pathways. *Life Sci.,* **2020**, *250*, 117591. http://dx.doi.org/10.1016/j.lfs.2020.117591

[7] Shah, M.A.; Adnan, M.; Rasul, A.; Hussain, G.; Sarfraz, I.; Nageen, B.; Riaz, A.; Khalid, R.; Asrar, M.; Selamoglu, Z.; Adem, Ş.; Sarker, S.D. Physcion and physcion 8-O-β-D-glucopyranoside: Natural anthraquinones with potential anti-cancer activities. *Curr. Drug Targets,* **2020**. Available from: ljmu.ac.uk

[8] Ozdemir, B.; Gulhan, M.F.; Sahna, E.; Selamoğlu, Z. The investigation of antioxidant and anti-inflammatory potentials of apitherapeutic agents on heart tissues in nitric oxide synthase inhibited rats *via* Nω-nitro-L-arginine methyl ester. *Clin. Exp. Hypertens.,* **2021**, *43*(1), 69-76. http://dx.doi.org/10.1080/10641963.2020.1806294 PMID: 32799699

[9] Talas, Z.S.; Ilknur, O.; Ozdemir, I.; Ciftci, O.; Cakir, O.; Gulhan, M.F.; Pasaoglu, O.M. Role of propolis on biochemical parameters in kidney and heart tissues against L-NAME induced oxidative injury in rats. *Clin. Exp. Hypertens.,* **2014**, *36*(7), 492-496. http://dx.doi.org/10.3109/10641963.2013.863322

[10] Hajam, Y.A.; Rani, R.; Ganie, S.Y.; Sheikh, T.A.; Javaid, D.; Qadri, S.S.; Pramodh, S.; Alsulimani, A.; Alkhanani, M.F.; Harakeh, S.; Hussain, A.; Haque, S.; Reshi, M.S. Oxidative stress in human pathology and aging: Molecular mechanisms and perspectives. *Cells*, **2022**, *11*(3), 552. http://dx.doi.org/10.3390/cells11030552 PMID: 35159361

[11] Barreiros, A.L.B.S.; David, J.M.; David, J.P. Oxidative stress: Relationship between generation of reactive species and organism defense. *New Chem.* **2006**, 29, 113-123. https://doi.org/10.1590/S1516-05722012000200010

[12] Z, S. The natural products and healthy life. *J. Tradit. Med. Clin. Naturop.*, **2018**, *7*(2)e146 http://dx.doi.org/10.4172/2573-4555.1000e146

[13] Bourchard, J.R.; Wyse, D.L.; Sheaffer, C.C.; Kauppi, K.L.; Fulcher, G.R.; Ehlke, N.J.; Biesboer, D.D.; Bey, R.F. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. *J. Med. Plant Res.*, **2008**, *2*(4), 81-93. https://www.researchgate.net/journal/Journal-of-Medicinal-Plant-Research-1996-0875

[14] Rüdiger, A.L.; Veiga-Junior, V.F. Chemodiversity of ursane- and oleanane-type triterpenes in Amazonian *Burseraceae oleoresins*. *Chem. Biodivers.,* **2013**, *10*(6), 1142-1153. http://dx.doi.org/10.1002/cbdv.201200315 PMID: 23776030

[15] Selamoglu, Z. Polyphenolic compounds in human health with pharmacological properties. *J. Tradit. Med. Clin. Naturop.,* **2017**, *6*(4) http://dx.doi.org/10.4172/2573-4555.1000e138

[16] Akalın, G.; Selamoglu, Z. Nutrition and foods for skin health. *J. Pharm. Care,* **2019**, *7*(1-2), 31-33. http://dx.doi.org/10.18502/jpc.v7i1-2.1620

[17] Maia, R.M.; Barbosa, P.R.; Cruz, F.G.; Roque, N.F.; Fascio, M. Triterpenes from *Protium heptaphyllum* March resin. (Bourseraceae): Characterization in binary mixtures. *New Chem*. **2000**, *23*(5), 623-626. https://doi.org/10.1590/S0100-40422000000500010.

[18] Bandeira, P.N.; Pessoa, O.D.L.; Trevisan, M.T.S.; Lemos, T.M.G. Secondary metabolites of *Protium heptaphyllum* March. *New Chem*. **2002**, *25* (6b), 1078-1080. https://doi.org/10.1590/S0100.

[19] Marques, D.D.; Sartori, R.A.; Lemos, T.L.G.; Machado, L.L. Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*. *Acta Amazonica*. **2010**, *40* (1). https://doi.org/10.1590/S0044-59672010000100029.

[20] Islam, M.R.; Kabir, M.F.; Alam, R.; Dhar, R.; Rana, M.N.; Islam, M.E.; Parvin, M.S.; Hossain, M.A. Sedative, membrane stability, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall. *Asian Pac. J. Trop. Dis.,* **2014**, *4*(2), S928-S933. http://dx.doi.org/10.1016/S2222-1808(14)60760-X

[21] PPMC.ORG. Aromatic and spice medicinal plants. **2015**. https://www.ppmac.org/content/amescla

[22] Biodiversity4all. Biodiversity Association for All. **2019**. Available from: https://www.biodiversity4all.org/photos/31615929

[23] Barbosa, K.B.F.; Costa, N.M.B.; Alfenas, R.C.G.; De Paula, S.O.; Minim, V.P.R.; Bressan, J. Oxidative stress: Concept, implications and modulatory factors. *Rev. Nutr*., **2010**, *23*(4), 629-643. http://dx.doi.org/10.1590/S1415-52732010000400013

[24] Phaniendra, A.; Jestadi, D.B.; Periyasamy, L. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian J. Clin. Biochem.,* **2015**, *30*(1), 11-26. http://dx.doi.org/10.1007/s12291-014-0446-0 PMID: 25646037

[25] Durairaj, V.; Hoda, M.; Shakya, G.; Babu, S.P.P.; Rajagopalan, R. Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pac. J. Trop. Med.,* **2014**, *7*, S398-S404. http://dx.doi.org/10.1016/S1995-7645(14)60265-0 PMID: 25312157

[26] Bender, D.A.; Free Radicals & Antioxidant Nutrients. In: Rodwell, V.W., Bender, D.A., Botham, K.M., Kennelly, P.J., Weil, P. eds. Harper's Illustrated Biochemistry, 31 Ed. McGraw Hill; 2018. Accessed Julho, **2022**. https://accesspharmacy.mhmedical.com/content.aspx?bookid=2386&sectionid=187836421

[27] Vanzin, S.B.; Camargo, C.P. *Understanding cosmeceuticals: Diagnoses and treatments*; São Paulo: Santos, **2008**, pp. 397.

[28] Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Oxidative stress: Harms and benefits for human health. *Oxid. Med. Cell. Longev.,* **2017**, *2017*, 1-13. http://dx.doi.org/10.1155/2017/8416763 PMID: 28819546

[29] Trevisan, P.; Silva Ávila, D. Induction of photoaging by ultraviolet radiation in *Caenorhabditis elegans* in a DAF-16 dependent pathway. *Age,* **2021**, *13*(3). https://periodicos.unipampa.edu.br/index.php/SIEPE/article/view/110652

[30] Maynard, S.; Schurman, S.H.; Harboe, C.; de Souza-Pinto, N.C.; Bohr, V.A. Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis,* **2008**, *30*(1), 2-10. http://dx.doi.org/10.1093/carcin/bgn250 PMID: 18978338

[31] Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; Abete, P. Oxidative stress, aging, and diseases. *Clin. Interv. Aging,* **2018**, *13*(13), 757-772. http://dx.doi.org/10.2147/CIA.S158513 PMID: 29731617

[32] Sies, H. On the history of oxidative stress: Concept and some aspects of current development. *Curr. Opin. Toxicol.,* **2018**, *7*, 122-126. http://dx.doi.org/10.1016/j.cotox.2018.01.002

[33] Singh, A.; Kukreti, R.; Saso, L.; Kukreti, S. Oxidative stress: A key modulator in neurodegenerative diseases. *Molecules,* **2019**, *24*(8), 1583. http://dx.doi.org/10.3390/molecules24081583 PMID: 31013638

[34] Huang, D.; Ou, B.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.,* **2005**, *53*(6), 1841-1856. http://dx.doi.org/10.1021/jf030723c PMID: 15769103

[35] Vieira, L.A.S.L.; Souza, R.B.A. Action of antioxidants in combating free radicals and preventing skin aging. id on line, *Rev. Mult. Psic.,* **2019**, *13*(48), 408-418. http://dx.doi.org/10.14295/idonline.v13i48.2210

[36] Salehi, B.; Martorell, M.; Arbiser, J.; Sureda, A.; Martins, N.; Maurya, P.; Sharifi-Rad, M.; Kumar, P.; Sharifi-Rad, J. Antioxidants: Positive or negative actors? *Biomolecules*, **2018**, *8*(4), 124. http://dx.doi.org/10.3390/biom8040124 PMID: 30366441

[37] Gulcin, İ. Antioxidants and antioxidant methods: An updated overview. *Arch. Toxicol.,* **2020**, *94*(3), 651-715. http://dx.doi.org/10.1007/s00204-020-02689-3 PMID: 32180036

[38] Kuriakose, G.C.; Kurup, M.G. Antioxidant and hepatoprotective activity of Aphanizomenon flos-aquae Linn against paracetamol intoxication in rats. *Indian J. Exp. Biol.,* **2010**, *48*(11), 1123-1130. PMID: 21117453

[39] Simões, C.M.O.; Schenkel, E.P.; Mello, J.C.P.D.; Mentz, L.A.; Petrovick, P.R. Pharmacognosy. Artemed; August 1999, 6 Ed. Publisher: Editors of the UFSC and UFRGS. Available in: https://www.researchgate.net/publication/315772641_Farmacognosia_da_Planta_ao_Medicamento.

[40] Blasco, A.J.; Rogerio, M.C.; González, M.C.; Escarpa, A. "Electrochemical Index" as a screening method to determine "total polyphenolics" in foods: A proposal. *Anal. Chim. Acta,* **2005**, *539*(1-2), 237-244. http://dx.doi.org/10.1016/j.aca.2005.02.056

[41] Oliveira, L.F.D.; Maior, J.F.A.S.; Dresch, R.R. Pure pharmacognosy. Porto Alegre. SAGAH. Grupo A., **2018**. Available from: https://sagah.maisaedu.com.br/

[42] Tsuchiya, H. Membrane interactions of phytochemicals as their molecular mechanism applicable to the discovery of drug leads from plants. *Molecules,* **2015**, *20*(10), 18923-18966. http://dx.doi.org/10.3390/molecules201018923 PMID: 26501254

[43] Agati, G.; Brunetti, C.; Di Ferdinando, M.; Ferrini, F.; Pollastri, S.; Tattini, M. Functional roles of flavonoids in photoprotection: New evidence, lessons from the past. *Plant Physiol. Biochem.,* **2013**, *72*, 35-45. http://dx.doi.org/10.1016/j.plaphy.2013.03.014 PMID: 23583204

[44] Rosa, L.A.; Moreno-Escamilla, J.O.; Rodrigo-García, J.; Alvarez-Parrilla, E. *Phenolic Compounds*; Postharvest Biol. Technol. of Fruits and Vegetables, **2019**, pp. 253-271. http://dx.doi.org/10.1016/B978-0-12-813278-4.00012-9

[45] Balasundram, N.; Sundram, K.; Samman, S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem.,* **2006**, *99*(1), 191-203. http://dx.doi.org/10.1016/j.foodchem.2005.07.042

[46] Lôbo, G.B.S.; Silvac, A.V.; Menezes, G.B.L. Dietary polyphenols and endothelial function in undiagnosed adults: A systematic review of randomized trials. *Braz. J. Dev.,* **2020**, *6*, 11. http://dx.doi.org/10.34117/bjdv6n11-085

[47] Badshah, S.L.; Faisal, S.; Muhammad, A.; Poulson, B.G.; Emwas, A.H.; Jaremko, M. Antiviral activities of flavonoids. *Biomed. Pharmacother.,* **2021**, *140*, 111596. http://dx.doi.org/10.1016/j.biopha.2021.111596

[48] Parham, S.; Kharazi, A.Z.; Bakhsheshi-Rad, H.R.; Nur, H.; Ismail, A.F.; Sharif, S.; RamaKrishna, S.; Berto, F. Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants,* **2020**, *9*(12), 1309. http://dx.doi.org/10.3390/antiox9121309 PMID: 33371338

[49] Pimentel, C.V.M.B.; Francki, V.M.; Gollücke, A.P.B. *Functional foods: Introduction to the main bioactive substances in foods*; Varela: São Paulo, **2005**.

[50] Pei, R.; Liu, X.; Bolling, B. Flavonoids and gut health. *Curr. Opin. Biotechnol.,* **2020**, *61*, 153-159. http://dx.doi.org/10.1016/j.copbio.2019.12.018 PMID: 31954357

[51] Devi, S.; Kumar, V.; Singh, S.K.; Dubey, A.K.; Kim, J.J. Flavonoids: Potential candidates for the treatment of neurodegenerative disorders. *Biomedicines,* **2021**, *9*(2), 99. http://dx.doi.org/10.3390/biomedicines9020099 PMID: 33498503

[52] Lalani, S.; Poh, C.L. Flavonoids as antiviral agents for enterovirus A71 (EV-A71). *Viruses,* **2020**, *12*(2), 184. http://dx.doi.org/10.3390/v12020184

[53] Russo, M.; Moccia, S.; Spagnuolo, C.; Tedesco, I.; Russo, G.L. Roles of flavonoids against coronavirus infection. *Chem. Biol. Interact.,* **2020**, *328* (328), 109211. http://dx.doi.org/10.1016/j.cbi.2020.109211 PMID: 32735799

[54] Lima, R.A. The importance of taxonomy, phytochemistry and bioprospection of vegetable species aiming at combating and facing covid-19. *SAJEBTT,* **2020**, *7*(1).
Available in: https://periodicos.ufac.br/index.php/SAJEBTT/article/view/3721. Accessed in 04 March 2022.

[55] Freitas, S.T.F.; Benvindo-Souza, M.; Teodoro, L.O.; Goulart, M.M.P.; Pinto, T.F.E.; Azevedo, M.O.; Valentim, A.M.; Pereira, P.S.; Santos, L.R.S.; Dyszy, F.H. Taxonomic aspects of bio-prospecting in Brazil: Scientific trend. *Oecol. Aust.,* **2020**, *24*(4), 770-780.
http://dx.doi.org/10.4257/oeco.2020.2404.02

[56] Oprean, C.; Ivan, A.; Bojin, F.; Cristea, M.; Soica, C.; Drăghia, L.; Caunii, A.; Paunescu, V.; Tatu, C. Selective *in vitro* anti-melanoma activity of ursolic and oleanolic acids. *Toxicol. Mech. Methods,* **2018**, *28*(2), 148-156.
http://dx.doi.org/10.1080/15376516.2017.1373881 PMID: 28868958

[57] Neuman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J. Nat. Prod.,* **2012**, *75*(3), 311-335.
http://dx.doi.org/10.1021/np200906s PMID: 22316239

[58] Rodrigues, A.C.B.C.; Oliveira, F.P.; Dias, R.B.; Sales, C.B.S.; Rocha, C.A.G.; Soares, M.B.P.; Costa, E.V.; Silva, F.M.A.; Rocha, W.C.; Koolen, H.H.F.; Bezerra, D.P. *In vitro* and *in vivo* anti-leukemia activity of the stem bark of *Salacia impressifolia* (Miers) A. C. Smith (Celastraceae). *J. Ethnopharmacol.,* **2019**, *231*, 516-524.
http://dx.doi.org/10.1016/j.jep.2018.11.008 PMID: 30445109

[59] Brito, A.F.; Ribeiro, M.; Abrantes, A.M.; Gonçalves, A.C.; Sarmento-Ribeiro, A.B.; Tralhão, J.G.; Botelho, M.F. Quercetin's anti-cancer action in hepatocellular carcinoma: The role of GLUT-1. *Portuguese J. Surg.,* **2013**, 2, 23-30.
https://revista.spcir.com/index.php/spcir/article/view/310

[60] Pereira, W.L.; Oliveira, T.T.; Kanashiro, M.; Costa, M.R. Antiproliferative action flavonoid morin and olive leaf extract (*Oleaeuropaea L.)* against the H460 cell line. *Braz. J. Med. Plants,* **2015**, *17*(1), 798-806.
http://dx.doi.org/10.1590/1983-084X/14_020

[61] Mousa, A.M.; El-Sammad, N.M.; Hassan, S.K.; Madboli, A.E.N.A.; Hashim, A.N.; Moustafa, E.S.; Bakry, S.M.; Elsayed, E.A. Antiulcerogenic effect of *Cuphea ignea* extract against ethanol-induced gastric ulcer in rats. *BMC Complement. Altern. Med.,* **2019**, *19*(1), 345.
http://dx.doi.org/10.1186/s12906-019-2760-9 PMID: 31791313

[62] Cappello, A.R.; Dolce, V.; Iacopetta, D.; Martello, M.; Fiorillo, M.; Curcio, R.; Muto, L.; Dhanyalayam, D. Bergamot (*Citrus bergamia* Risso) flavonoids and their potential benefits in human hyperlipidemia and atherosclerosis: An overview. *Mini Rev. Med. Chem.,* **2016**, *16*(8), 619-629.
http://dx.doi.org/10.2174/1389557515666150709110222 PMID: 26156545

[63] Oliveira, F.A.; Costa, C.L.S.; Chaves, M.H.; Almeida, F.R.C.; Cavalcante, Í.J.M.; Lima, A.F.; Lima, R.C.P., Jr; Silva, R.M.; Campos, A.R.; Santos, F.A.; Rao, V.S.N. Attenuation of capsaicin-induced acute and visceral nociceptive pain by α- and β-amyrin, a triterpene mixture isolated from *Protium heptaphyllum* resin in mice. *Life Sci.,* **2005**, *77*(23), 2942-2952.
http://dx.doi.org/10.1016/j.lfs.2005.05.031 PMID: 15964027

[64] Nogueira, A.O.; Oliveira, Y.I.S.; Adjafre, B.L.; de Moraes, M.E.A.; Aragão, G.F. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from *Protium heptaphyllum* : A literature review. *Fundam. Clin. Pharmacol.,* **2019**, *33*(1), 4-12.
http://dx.doi.org/10.1111/fcp.12402 PMID: 30003594

[65] Citó, A.G.M.L.; Costa, F.B.; Lopes, J.A.D.; Oliveira, V.M.M.; Chaves, M.H. Identification of volatile constituents of fruits and leaves of *Protium heptaphyllum* Aubl (March). *Braz. J. Med. Plants,* **2006**, *8*(4), 4-7.

[66] Domene, V.D.; Matos, P.P.; Salis, S.M. Growth of *Protium heptaphyllum* in the Pantanal of Nhecolândia, Mato Grosso do Sul. 5th Symposium on Socio-Economic Natural Resources of the Wetland. 2010. From 9 to 12 November 2010. **2010**.
Available from: https://www.alice.cnptia.embrapa.br/handle/doc/869564?locale=en. Accessed on June 10, 2022.

[67] Costa, T.O.G.; Almeida, R.A.; Koolen, H.H.F.; Silva, F.M.A.; Pinto, A.C. Constituintes químicos do caule de *Protium hebetatum* (Burseraceae). *Acta Amazônia,* **2012**, *42*(4), 557.
https://www.scielo.br/j/aa/a/YGfqWBJyWfrtJNbXFLZ6YLF/?format=pdf&lang=pt

[68] Bandeira, P.N.; Fonseca, A.M.; Costa, S.M.O.; Lins, M.U.D.S.; Pessoa, O.D.L.; Monte, F.J.Q.; Nogueira, N.A.P.; Lemos, T.L.G. Anti-bacterial and antioxidant activities of the essential oil of resin of *Protium heptaphyllum*. *Nat. Prod. Commun.,* **2006**, *1*(2)1934578X0600100
http://dx.doi.org/10.1177/1934578X0600100207

[69] Almeida, E.X.; Conserva, L.M.; Lyra Lemos, R.P. Coumarins, coumarinolignoids and terpenes from *Protium heptaphyllum*. *Biochem. Syst. Ecol.,* **2002**, *30*(7), 685-687.
http://dx.doi.org/10.1016/S0305-1978(01)00130-2

[70] Albino, R.C.; Oliveira, P.C.; Prosdocimi, F.; da Silva, O.F.; Bizzo, H.R.; Gama, P.E.; Sakuragui, C.M.; Furtado, C.; de Oliveira, D.R. Oxidation of monoterpenes in *Protium heptaphyllum* oleoresins. *Phytochemistry,* **2017**, *136*, 141-146.
http://dx.doi.org/10.1016/j.phytochem.2017.01.013 PMID: 28143669

[71] Tafurt-García, G.; Muñoz-Acevedo, A. Metabolitos volátiles presentes em *Protium heptaphyllum* (Aubl.) March. colectado en Tame (Arauca-Colombia). *Bol. Latinoam. Caribe Plantas Med. Aromat.,* **2012**, *11*(3), 223-232.
https://pesquisa.bvsalud.org/portal/resource/pt/lil-647661

[72] Holanda Pinto, S.A.; Pinto, L.M.S.; Cunha, G.M.A.; Chaves, M.H.; Santos, F.A.; Rao, V.S. Anti-inflammatory effect of α, β-Amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. *Inflammopharmacol,* **2008**, *116*, 48-52.
http://dx.doi.org/10.1007/s10787-007-1609-x

[73] Aragão, G.F.; Carneiro, L.M.V.; Junior, A.P.F.; Vieira, L.C.; Bandeira, P.N.; Lemos, T.L.G.; Viana, G.S.B. A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacol. Biochem. Behav.,* **2006**, *85*(4), 827-834.
http://dx.doi.org/10.1016/j.pbb.2006.11.019 PMID: 17207523

[74] Carvalho, K.M.; Marinho Filho, J.D. T.S.; Melo, A.J.; Araújo, J.S.; Quetz, M.P.; Cunha, K.M.; Melo, A.A.; Silva, A.R.; Tomé, A.; Havt, S.G.; Fonseca, G.A.; Brito, M.H.; Chaves, V.S.; Rao, F.A.; The resin from *Protium heptaphyllum* prevents high-fat diet-induced obesity in mice: Scientific evidence and potential mechanisms. *Evid. Based Complement. Alternat. Med.,* **2015**, *53*(3), 407-413.
http://dx.doi.org/10.1155/2015/106157 PMID: 25709707

[75] Oliveira, F.; Vieira-Júnior, G.M.; Chaves, M.H.; Almeida, F.R.; Florêncio, M.G.; Lima, R.C., Jr; Silva, R.M.; Santos, F.A.; Rao, V.S. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. *Pharmacol. Res.,* **2004**, *49*(2), 105-111.
http://dx.doi.org/10.1016/j.phrs.2003.09.001 PMID: 14643690

[76] Cabral, R.S.C.; Alves, C.C.F.; Batista, H.R.F.; Sousa, W.C.; Abrahão, I.S.; Crotti, A.E.M.; Santiago, M.B.; Martins, C.H.G.; Miranda, M.L.D. Chemical composition of essential oils from different parts of *Protium heptaphyllum* (Aubl.) Marchand and their *in vitro* antibacterial activity. *Nat. Prod. Res.,* **2020**, *34*(16), 2378-2383.
http://dx.doi.org/10.1080/14786419.2018.1536659 PMID: 30499330

[77] Patias, N.S.; Gindri Sinhorin, V.D.; de Moura, F.R.; da Cunha, A.P.S.; da Silva Lima, R.R.; da Costa, T.B.; da Costa, R.J.; Cavalheiro, L.; de Campos Bicudo, R.; Sinhorin, A.P. Identification of Flavonoids by LC-MS/MS in leaves extract from *Protium heptaphyllum* (Aubl.) March and antioxidant activity in mice. *Nat. Prod. J.,* **2021**, *11*(5), 715-727.
http://dx.doi.org/10.2174/2210315510999200817165311

[78] Aragão, G.F.; Pinheiro, M.C.C.; Bandeira, P.N.; Lemos, T.L.G.; Viana, G.S.B. Analgesic and anti-inflammatory activities of the isomeric mixture of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *J. Herb. Pharmacother.,* **2008**, *7*(2), 31-47.
http://dx.doi.org/10.1080/J157v07n02_03 PMID: 18285306

[79] Aragão, G.F.; Carneiro, L.M.V.; rota-Junior, A.P.; Bandeira, P.N.; Lemos, T.L.G.; Viana, G.S.B. Alterations in brain amino acid metabolism and inhibitory effects on PKC are possibly correlated with anticonvulsant effects of the isomeric mixture of α- and β-amyrin from *Protium heptaphyllum*. *Pharm. Biol.,* **2015**, *53*(3), 407-413.
http://dx.doi.org/10.3109/13880209.2014.923001 PMID: 25471298

[80] Carvalho, K.M. Amyrins from *Protium heptaphyllum* reduce high-fat diet-induced obesity in mice *via* modulation of enzymatic, hormonal and inflammatory responses. *Planta Medica,* **2016**, *83*(3-4), 285-291.
http://dx.doi.org/10.1055/s-0042-114222

[81] Santos, F.A.; Frota, J.T.; Arruda, B.R.; de Melo, T.S.; da Silva, A.A.C.A.; Brito, G.A.C.; Chaves, M.H.; Rao, V.S. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis.,* **2012**, *11*(1), 98.
http://dx.doi.org/10.1186/1476-511X-11-98 PMID: 22867128

[82] Melo, C.M.; Morais, T.C.; Tomé, A.R.; Brito, G.A.C.; Chaves, M.H.; Rao, V.S.; Santos, F.A. Anti-inflammatory effect of α,β-amyrin, a triterpene from *Protium heptaphyllum*, on cerulein-induced acute pancreatitis in mice. *Inflamm. Res.,* **2011**, *60*(7), 673-681.
http://dx.doi.org/10.1007/s00011-011-0321-x PMID: 21400110

[83] Araújo, D.A.O.V.; Takayama, C.; de-Faria, F.M.; Socca, E.A.R.; Dunder, R.J.; Manzo, L.P.; Luiz-Ferreira, A.; Souza-Brito, A.R.M. Gastroprotective effects of essential oil from *Protium heptaphyllum* on experimental gastric ulcer models in rats. *Rev. Bras. Farmacogn.,* **2011**, *21*(4), 721-729.
http://dx.doi.org/10.1590/S0102-695X2011005000117

[84] Mannino, G.; Iovino, P.; Lauria, A.; Genova, T.; Asteggiano, A.; Notarbartolo, M.; Porcu, A.; Serio, G.; Chinigò, G.; Occhipinti, A.; Capuzzo, A.; Medana, C.; Munaron, L.; Gentile, C. Bioactive triterpenes of *Protium heptaphyllum* gum resin extract display cholesterol-lowering potential. *Int. J. Mol. Sci.*, **2021**, *22*(5), 2664.
http://dx.doi.org/10.3390/ijms22052664 PMID: 33800828

[85] Mendes, J.L.; de Araújo, T.F.; Geraldo de Carvalho, M.; Aragão Catunda Júnior, F.E.; Albuquerque Costa, R. Chemical composition and mechanism of vibriocidal action of essential oil from resin of *Protium heptaphyllum*. *Scientific World Journal,* **2019**, *2019*, 1-6.
http://dx.doi.org/10.1155/2019/9563213 PMID: 31780877

[86] Mobin, M.; De Lima, S.G.; Almeida, L.T.G.; Takahashi, J.P.; Teles, J.B.; Szeszs, M.W.; Martins, M.A.; Carvalho, A.A.; Melhem, M.S.C. MDGC-MS analysis of essential oils from *Protium heptaphyllum* (Aubl.) and their antifungal activity against Candida specie. *Rev. Bras. Plantas Med.,* **2016**, *18*(2), 531-538.
http://dx.doi.org/10.1590/1983-084X/15_110

[87] Faustino, C.G.; Medeiros, F.A.; Galardo, A.K.R.; Rodrigues, A.B.L.; Martins, R.L.; Lima, Y.M.S.; Tavares, J.F.; Medeiros, M.A.A.; Cruz, J.S.; Almeida, S.S.M.S. Larvicide activity on aedesaegypti of essential oil nanoemulsion from the *Protium heptaphyllum* resin. *Molecules,* **2020**, *25*(22), 5333.
http://dx.doi.org/10.3390/molecules25225333

[88] Endringer, D.C.; de Lima, E.M.; Cazelli, D.S.P.; Pinto, F.E.; Mazuco, R.A.; Kalil, I.C.; Lenz, D.; Scherer, R.; de Andrade, T.U. Essential oil from the resin of *Protium heptaphyllum*: Chemical composition, cytotoxicity, antimicrobial activity, and antimutagenicity. *Pharmacogn. Mag.,* **2016**, *12*(45)(Suppl. 1), 42.
http://dx.doi.org/10.4103/0973-1296.176113 PMID: 27041857

[89] Bernadi, W.A.; Zanotelli, J.C.; Lima, E.M.; Souza, T.D.; Edringer, D.C.; Souza, V.R.C. Effects of topical application of essential oil from resin of almescar *Protium heptaphyllum* (Aubl.) Marchand in experimentally induced skin wounds in rats. *Rev. Bras. Cienc. Vet.,* **2015**, *22*(1), 10-15.
http://dx.doi.org/10.4322/rbcv.2015.311

[90] Mobin, M.; de Lima, S.G.; Almeida, L.T.G.; Silva Filho, J.C.; Rocha, M.S.; Oliveira, A.P.; Mendes, M.B.; Carvalho, F.A.A.; Melhem, M.S.C.; Costa, J.G.M. Gas chromatography-triple quadrupole mass spectrometry analysis and vasorelaxant effect of essential oil from *Protium heptaphyllum* (aubl.) March. *Bio. Med. Res. Int.,* **2017**, *2017*, 1-6.
http://dx.doi.org/10.1155/2017/1928171 PMID: 28951867

[91] Oliveira, F.A.; Chaves, M.H.; Almeida, F.R.C.; Lima, R.C.P., Jr; Silva, R.M.; Maia, J.L.; Brito, G.A.A.C.; Santos, F.A.; Rao, V.S. Protective effect of α- and β-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.,* **2005**, *98*(1-2), 103-108.
http://dx.doi.org/10.1016/j.jep.2005.01.036 PMID: 15763370

[92] Pontes, W.J.T.; Oliveira, J.C.G.; Câmara, C.A.G.; Lopes, A.C.H.R.; Godim Júnior, M.G.C.; Oliveira, J.V.; Barros, R.; Schwartz, M.O.E. Chemical composition and acaricidal activity of the leaf and fruit essential oils of *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae). *Acta Amazonica* **2013**, *37*(1).
https://doi.org/10.1590/S0044-59672007000100012.

[93] Wojtunik-Kulesza, K.; Oniszczuk, A.; Oniszczuk, T.; Combrzyński, M.; Nowakowska, D.; Matwijczuk, A. Influence of *in vitro* digestion on composition, bioaccessibility and antioxidant activity of food polyphenols-A non-systematic review. *Nutrients,* **2020**, *12*(5), 1401.
http://dx.doi.org/10.3390/nu12051401 PMID: 32414132

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CAPÍTULO 2

Effect of Liposomal *Protium heptaphyllum* (Alb.) March Extract in the Treatment of Obesity Induced by High-Calorie Diet

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Effect of Liposomal *Protium heptaphyllum* (Alb.) March Extract in the Treatment of Obesity Induced by High-Calorie Diet

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Simple Summary: Obesity is a chronic disease caused by excessive consumption of high-calorie foods that is associated with conditions such as dyslipidemia, diabetes, and cancer. *Protium heptaphyllum* (*P. heptaphyllum*) is known in folk medicine for its analgesic, anti-inflammatory, and healing properties, and its resin has demonstrated anti-obesity effects. This study aimed to evaluate the impact of liposomes containing the ethyl acetate fraction from *P. heptaphyllum* leaves on obesity in male Wistar rats. The animals became obese through a high-calorie diet and were treated with a liposome formulation containing *P. heptaphyllum* for 14 days. The study evaluated several parameters in adipose and liver tissues. Despite some positive effects on liver function and inflammation, the treatment did not attenuate global changes related to obesity, such as weight gain and fat accumulation. This highlights the complexity of obesity treatment and the varied responses of different organs to *P. heptaphyllum* liposomes.

Abstract: Obesity, a chronic disease, resulted from excessive consumption of high-calorie foods, leading to an energy imbalance. *Protium heptaphyllum* (*P. heptaphyllum*) was used in folk medicine for its analgesic, anti-inflammatory, and healing properties. The association of the extract from *P. heptaphyllum* with nanotechnology was innovative for combining high technology with active ingredients that are easily accessible in the Amazon region. This study evaluated the effect of liposomes containing the ethyl acetate fraction of the crude extract of *P. heptaphyllum* leaves on obesity. Male Wistar rats treated with a high-calorie diet for 8 weeks to induce obesity received treatment with the liposome formulation containing *P. heptaphyllum* extract (1 mg/kg/day, via gavage) for 14 days. Morphological, metabolic, redox status, immunological, and histological parameters were evaluated in the adipose and liver tissue of the animals. The groups were divided as follows: C: control; P: liposomes containing extract; O: obese, and OP: obese + liposomes containing extract. The obesity model resulted in increases in body weight, caloric intake, body fat weight, and in the lipid profile. In adipose tissue, P decreased SOD (superoxide dismutase) activity in obese animals. In the liver, a positive modulation of the extract was observed in relation to glucose, amino acids, lactate, hepatoprotective action, and anti-inflammatory activity, with a decrease in interleukin 1β (IL-1β) in obese animals. The results showed an improvement in the functional and inflammatory aspects, but the treatment was not effective in alleviating general changes related to obesity, such as weight gain, fat, glucose, triglycerides, and inflammation in adipose tissue, highlighting the complexity of responses in different organs during obesity and treatment with *P. heptaphyllum*.

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Keywords: obesity; *Protium heptaphyllum*; cytokines; oxidative stress; metabolism

1. Introduction

Obesity is prevalent and growing, affecting all ages, and is considered a significant public health problem [1,2]. It results from energy imbalance, especially due to excessive consumption of high-calorie foods [3]. Furthermore, it is directly associated with metabolic syndrome, with oxidative stress as one of the main triggers of complications, including dyslipidemia, diabetes, hypertension, musculoskeletal disorders, and cancer [4,5]. The biochemical mechanisms involved include nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase activation, oxidative phosphorylation, glyceraldehyde auto oxidation, protein kinase C activation, polyol and hexosamine pathways, hyperleptinemia, low antioxidant defense, postprandial reactive oxygen species generation, and chronic inflammation [6].

Chronic inflammation in adipose tissue produces pro-inflammatory chemokines and cytokines, stimulating immune cells from circulation to adipose tissue [7]. Obesity increases pro-inflammatory adipokines, such as interleukin-6 (IL-6), Alpha Tumor Necrosis Factor (TNF-α), adiposin, angiotensinogen, leptin, resistin, and visfatin, while decreasing the expression of anti-inflammatory adipokines, such as interleukin-10 (IL-10) and adiponectin, both in the systemic circulation and in tissues [8].

Herbal medicines offer an effective, affordable solution with less side effects to treat these disorders [9]. The use of these medications, which control oxidative stress and balance lipids, has been widely tested in therapeutic interventions [10]. However, more studies are needed to investigate the biological activity and toxicity of plants, aiming to attract investment from the pharmaceutical industry [9].

Nanotechnology has revolutionized the pharmaceutical sector, especially with advances in the use of liposomes (lipid vesicles), for more effective drug delivery [11]. Liposomes are vesicles formed by one or more phospholipid bilayers oriented concentrically around an aqueous compartment. Liposomes offer advantages over other nanosystems, such as their ability to encapsulate hydrophilic and lipophilic drugs depending on the affinity of the incorporated substance [12,13]. Liposomes can be produced on a nano- or micrometric scale, depending on the manufacturing technique [14]. Obtaining these vesicles incurs low manufacturing costs and is easily scalable compared to polymeric nanocapsules, for example. These promising liposomes contribute to safer and more effective medicines [15], especially when combined with plant extracts, expanding their therapeutic possibilities. This convergence between nanotechnology, liposomes, and plant extracts marks an evolution in pharmacology, facilitating the development of personalized and sustainable medicines [16,17]. This is because the encapsulation and delivery of flavonoids has resulted in the design of an innovative liposomal encapsulation technology to effectively deliver flavonoids to specific cellular targets and organelles [18].

Plant substances are promising sources for new medicines [19,20]. *Protium heptaphyllum* (*P. heptaphyllum*), known as "almacega" or "breu blanco", is native to the Amazon [19–21] and is used in folk medicine for its stimulating, anti-inflammatory, and healing properties. Belonging to the Burseraceae family, with 18 genera and more than 700 species, the genus *Protium* is the main member [22]. Rich in triterpenes and flavonoids, *P. heptaphyllum* resin has demonstrated several bioactive properties, such as anti-inflammatory, antidepressant, anti-obesity, gastroprotective, and antibacterial actions [23–27]. Previous studies have investigated the effect of *P. heptaphyllum* leaf extracts against oxidative stress in mice, demonstrating antioxidant, hepatoprotective, and hypoglycemic properties [28].

Bioprospecting for natural products offers significant benefits to humanity, as biodiversity represents a valuable genetic library with future costs and benefits not yet fully known, with the Plantae kingdom being a crucial source of traditional medicine and an excellent reservoir for the discovery of new compounds with effective bioactives against difficult-to-treat diseases [29]. Thus, considering the anti-obesity effect already described

by the resin and the antioxidant effect identified by the plant's leaves, and knowing that there is an association between obesity and oxidative stress, this study aimed to demonstrate the therapeutic potential of the ethyl acetate fraction from the crude extract of *P. heptaphyllum* leaves, which are rich in flavonoids, in liposome form in an induced obesity model, according to a study by Patias et al. [28].

2. Material and Methods

2.1. Extract Preparation

The work was developed at the Integrated Chemical Sciences Research Laboratories (LIPEQ, Natural Products Chemistry Laboratory and Biochemistry Laboratory) at the Federal University of Mato Grosso, Campus of Sinop/UFMT. The exsicata is deposited in the collection of the Herbarium Centro-Norte-Mato-Grossense (CNMT) of the Federal University of Mato Grosso, Sinop campus, under number 625.

The leaves of *P. heptaphyllum* were selected and dried, crushed to powder, and then macerated with ethanol for seven days. Chlorophyll was removed with activated charcoal, then the extract was rotary-evaporated and lyophilized to be subjected to tests to identify functional groups. Part of the extract was fractionated in a silica gel chromatographic column with a gradient of solvents of increasing polarity, procedures that are more detailed in Reference [28]. After fractionation, the ethyl acetate fraction from *P. heptaphyllum* was chosen to be used in the development of liposomes, as it presented a greater amount of flavonoids.

2.1.1. Development of Liposomes

Liposomes containing the active ingredient were developed using the reversed-phase evaporation method [30], followed by extrusion of the lipid film. First, soy phosphatidylcholine (sPC) and cholesterol (COL) were diluted in chloroform in a molar ratio of 4:1. The aqueous phase was prepared containing phosphate-buffered saline (pH 7.4) and polysorbate 80 and kept in ultrasound for 5 min. Subsequently, 1 mL of the aqueous phase was added to the organic phase and kept under ultrasound (5 min), and the organic solvent was removed by rotary evaporation at 25 °C until the formation of a thin film. This film was hydrated with the remainder of the aqueous phase and kept under stirring for 30 min. Finally, the liposome was subjected to a micelle extrusion process with 10 extrusion cycles using polycarbonate membranes with pores of decreasing size. The liposome containing the *P. heptaphyllum* extract will be called $LP_{EB,}$ and the liposome without the active ingredient will be called LP_{BR}.

2.1.2. Physicochemical Characterization of Liposomes

The liposomes were characterized regarding their active content, the average diameter of the nanoparticles, polydispersity index (PDI), zeta potential, and pH. The formulations were stored at room temperature and protected from light for 30 days. The analyses were described with the mean and standard deviation of 3 batches.

2.1.3. Particle Size Determination by Laser Diffractometry

The formulations were evaluated for mean equivalent sphere diameter (d4.3) and particle size distribution (Span) by laser diffraction using a Mastersizer® 2000 (Malvern Instruments, Malvern, UK). The analyses, obtained at 25 °C from a small volume of the sample (enough to obscure the device between 2 and 8), were carried out in approximately 100 mL of distilled water as a dispersing medium.

2.1.4. Particle Size Determination by Photon Correlation Spectroscopy

The average cumulant diameter (Z-average) and the polydispersity index (PDI), resulting from the application of the cumulant method, were measured at a detection angle of 173° (25 °C), by photon correlation spectroscopy analysis (Zetasizer® NanoZS model ZEN 3600, Malvern Instruments, Malvern, UK) at 25 °C. The liposomes were diluted 500 times (*v*:*v*) in ultrapure water, filtered (0.45 µm membrane), and analyzed using the average of 3 replications.

2.1.5. Analysis of pH and Zeta Potential

pH determination was carried out using a potentiometer (Denver® Instrument VB-10, New York, NY, USA) previously calibrated with pH 4.0 and 7.0 standards directly in the formulations. The results represent the average of three determinations. The zeta potential was determined after diluting the liposomes 500 times in a 10 $mM \cdot L^{-1}$ NaCl solution, previously filtered through a 0.45 µm membrane, with measurements being carried out in triplicate, analyzing the sample for its electrophoretic mobility.

2.1.6. Identification and Measurement of Quercetrin in the Liposome

Previous work by the group with the extract of *Protium heptaphyllum* (Alb. March) identified and quantified the flavonoids quercetin, quercetin-3-β-D-glucoside, myricetin, and phenolic compounds [28]. In the present work, we identified another flavonoid, quercetrin. The analysis of this flavonoid was carried out using high-performance liquid chromatography with UV detection (HPLC-UV, Perkin Elmer®, Waltham, MA, USA). The analytical study was carried out using high-performance liquid chromatography (HPLC) on a Perkin Elmer Series 200 chromatograph (Cotati, Sonoma, CA, USA), equipped with an ultraviolet detector. The system consisted of a LiChrosorb 100 RP-18 stainless steel column (5 µm, 250 × 4 mm) and a pre-column with LiChrosorb RP-18 stationary phase (5 µm). The mobile phase consisted of a mixture of acetonitrile: water (90:10 v/v), and the flow rate used was 0.7 mL/min.

The dosage of quercetrin contained in the liposome was carried out in triplicate, and the method was validated according to Harmonization of Technical Requirements of Registration of Pharmaceutical for Humane Use and Resolution No. 899 of the National Health Surveillance Agency. The methodology was validated in terms of specificity, linearity, precision, accuracy, limits of detection, and quantification.

2.2. In Vivo Tests

2.2.1. Malone Test

Firstly, Malone's Hippocratic test was performed to create a dose curve of the LP_{EB} (0.5, 1.0, 2.5, and 5 mg/kg, using three animals for each concentration) to analyze the possible acute toxicity of the doses and the lethal dose [31]. From there, a dose was selected to administer to the animals.

2.2.2. Animals and Treatment

For the animal study, male Wistar rats were used. All steps to authorize work with animals were approved by the Guidelines of the Ethics Committee on the Use of Animals of the Federal University of Mato Grosso no. 23108.031684/2021-21. Animals were used in numbers of eight per group over a 14-day treatment period.

Control animals were fed standard rodent chow (NUVILAB CR-1, Nuvital®, Colombo, Paraná, Brazil), and obese animals received hypercaloric chow (rich in lipids; 24.5% of energy coming from lipids) and water with sucrose (300 g/L). The model and composition of the diet were prepared according to References [32,33]. The high-calorie diet was developed in the laboratory and contained commercial food (NUVILAB CR-1), condensed milk, cornstarch biscuits, casein, lard, vitamins, and minerals (Table 1). The ingredients were first ground and then mixed with vitamins and minerals. The mixture was transformed into pellets, dried in a drying oven at 45 ± 5 °C for 48 h, turned after 24 h, and stored at −8 °C.

Table 1. Composition of standard feed and high-calorie feed.

Components	Standard Ration	Hypercaloric Ration
Carbohydrates (%)	65.5	45.2
Proteins (casein > 99%) (%)	22	20.9
Lipids (%)	4	24.5
Fibers (%)	4	4
Vitamins * (%)	1	1
Mixture of minerals * (%)	3.5	3.5
Total (%)	100	100
Caloric value (kcal)	3.800	4.849

* Supplemented content of vitamins and minerals per 1000 g of high-calorie feed: iron: 25.2 mg; potassium: 104.8 µg; selenium: 73.1 µg; molybdenum sulfate: 150.0 µg; vitamin B_{12}: 34.5 µg; vitamin B_6: 6 µg; biotin: 0.12 µg; vitamin E: 48.9 IU; vitamin D: 2447.0 IU; and vitamin A: 15291.2 IU.

Throughout the experimental period, the animals were kept in polypropylene boxes, placed in an environment with a controlled temperature of 22 ± 2 °C and a 12 h light–dark cycle, with free access to water and food. The animals were distributed into 4 experimental groups according to whether they were treated with LP_{EB}. We identified the groups as follows:

Group C: Control group;
Group P: Control group treated with LP_{EB};
Group O: Obese group
Group OP: Obese group treated with LP_{EB}.

The acclimatization period was two weeks. The groups treated with LP_{EB} (P, OP) received the treatment dose defined by the Malone test [31], which was 1.0 mg/kg via gavage, once a day for 14 days. The control groups (C and O) received vehicle (water—1 mL/kg) via gavage also during the 14 days.

After the treatment period, the animals were fasted for 8 h. Blood was collected by cardiac puncture under anesthesia with Ketamine 50 mg/kg and Xilaxin 2 mg/kg, then the animals were euthanized by decapitation. Liver and adipose tissue samples were collected by dissection, washed with isotonic saline, and weighed to determine absolute (g) and relative weight (g/100 g of body weight). Furthermore, the weight of the mice (g) was evaluated, and tissue samples were stored frozen at −80 °C for later analysis, except for tissue samples collected for histological analyses, which were separated and processed immediately. Plasma was obtained from whole blood by centrifugation.

2.2.3. Characterization of Obesity and Analysis of the Metabolic Parameters

To characterize and confirm the effectiveness of the model for inducing obesity using a high-calorie diet, obesity was assessed by determining the Adiposity Index = [(periepididymal fat + retroperitoneal fat)/body weight × 100]; the relative weight of periepididymal and retroperitoneal fats, and lean mass (soleus and EDL muscles) in grams/100 g of body weight.

Weight gain and water and feed intake were assessed from the 1st to the 8th week.

2.2.4. Feed and Water Consumption

To evaluate daily food consumption, the rats were housed in polypropylene boxes and provided with free access to water and food. Initially, 500 g of food was placed in each box. After a period of 48 or 72 h (on weekends), the remaining amount of food was weighed. The difference obtained represented the volume consumed by the rats in the box, which was then divided by the number of animals present and the interval of 2 or 3 days (corresponding to the period between weighing). This procedure was repeated throughout the experiment, allowing for the evaluation of feed consumption from the beginning to the end of the treatment. The results were expressed in grams. The protocol used to analyze water consumption followed the same procedure adopted for feed consumption, considering

that the amount of water supplied to the animals every two or three days was 1000 mL. The animals' food consumption expressed in calories was obtained using a mathematical formula: group C and P: (amount consumed in food/day/rat (g) × 3.77 (kcal)) + (amount consumed in water/day/rat (mL) × 0 (kcal)) = value consumed in calories per day per rat (kcal); group O and OP: (amount consumed in food/day/rat (g) × 5.14 (kcal)) + (amount consumed in water/day/rat (mL) × 1.2 (kcal)) = amount consumed in calories per day per rat (kcal). The caloric value (kcal) of 1 g of feed was 3.77 kcal, that of 1 g of high-fat feed was 5.25 kcal, and 1.20 kcal for 1 mL of sugar-water (sucrose) solution [33].

2.2.5. Intraperitoneal Insulin Tolerance Test (IPITT) and Oral Glucose Tolerance Test (OGTT)

For the Intraperitoneal Insulin Tolerance Test (IPITT), caudal blood samples were collected before and at 4, 8, 12, 16, and 20 min after an intraperitoneally injected regular insulin overload. The constant rate of disappearance of blood glucose during the test (KITT) was calculated based on linear regression of the Neperian logarithm of glucose concentration. The animals were evaluated on the tenth day of treatment.

In the Oral Glucose Tolerance Test (OGTT), a caudal blood sample was collected and corresponded to basal glycemia (T0), after a period of 15 h of food restriction. Next, glucose was administered at a dose of 2.5 g/kg of body weight via gavage (0.5 g/mL glucose solution). Afterward, blood samples were collected at 15, 30, 60, 90, and 120 min after glucose administration corresponding to T15′, T30′, T60′, T90′, and T120′. Blood glucose was determined using a glucometer and strips (SENS II® Glucometer, Injex, Brazil). The result was calculated using the value of the area under the curve, represented by glucose [(mg/dL × min^{-1}) × 1000].

2.2.6. Biochemical Analyses in Liver, Adipose Tissue, and Plasma

The dosages of enzymatic antioxidant activity of CAT (catalase) were determined according to Reference [34], SOD (superoxide dismutase) according to Reference [35], GST (glutathione-S-transferase) according to Reference [36], and GPx (glutathione peroxidase) was measured according to Reference [37]. The dosage of the non-enzymatic antioxidant GSH (reduced glutathione) was carried out according to Reference [38], and that of ascorbic acid (AAS) was measured according to Reference [39]. The indirect markers of oxidative damage evaluated were TBARS (substances reactive to thiobarbituric acid) and carbonyl (carbonylated proteins) according to the technique described by References [40,41], respectively. The protein content of the tissues, except for the ascorbic acid dosage, was determined according to Reference [42].

The biochemical parameters (glucose, aspartate aminotransferase—AST, alanine aminotransaminase—ALT, alkaline phosphatase—ALP, amylase, lipase, creatinine, lactate and total proteins) and the lipid profile (total cholesterol, triglycerides, LDL—low-density lipoprotein, HDL—high-density lipoprotein) from blood plasma were analyzed at a partner Clinical Analysis Laboratory using a biochemical analyzer (XT-18000 Sysmex, Roche, Hitachi Ltd., Tokyo, Japan).

Glucose was determined according to Reference [43], glycogen according to Reference [44], and lactate was estimated according to Reference [45]. The protein content was determined by the method described in Reference [42], the amino acid dosage was carried out according to Reference [46], and ammonia was measured according to the reference method in [47].

2.2.7. Histological Analyses of Liver Tissue

For histological analyses of the liver, samples of liver tissue were fixed in buffered formalin and dehydrated in various concentrations of ethanol, then immersed in resin, sectioned into 3 µm transverse sections, and stained with hematoxylin and eosin (H&E), visualized under an optical microscope, and photographed as described by a previous study [48]. Liver structures were evaluated for hepatic inflammation, steatosis, hepatocyte degeneration, and fibrosis.

2.2.8. Immunological Analysis by ELISA

The homogenate obtained by diluting and homogenizing 50 mg of each tissue in phosphate-buffered saline (PBS) was centrifuged, and the supernatant was separated for use in measuring cytokines. The cytokines IL-6, IL-10, TNF-α, interferon gamma (IFN-γ), interleukin 17 (IL-17), and interleukin 1β (IL-1β) were measured according to commercial kits from R&D System, by sandwich ELISA technique. The concentrations of each cytokine were calculated based on the linear regression equation of the standard curve obtained with recombinant rat cytokines.

2.3. Data Analysis

Initially, the data passed the Kolmogorov–Smirnov normality test. The ANOVA (two-way) analysis of variance test was performed followed by the Tukey–Kramer multiple comparison test for comparing more than two means. Furthermore, in cases where the results did not pass the normality test, the Kruskal–Wallis's test and Dunn's post-test were performed. The minimum acceptable significance level was $p < 0.05$. The results were expressed as mean +/− standard deviation and/or median and total range. The graphs were generated and the results analyzed using the Graph Pad Prism 8.0 statistical program.

3. Results

3.1. Development and Characterization of Liposomes

The liposomes resulted in formulations with a homogeneous macroscopic, milky, opalescent, and slightly greenish appearance, a characteristic expected with the presence of *P. heptaphyllum* extract (LP_{EP}), and the formulations without the extract presented a whitish color (LP_{BR}). Table 2 presents the values referring to the average diameter, span, pH, and zeta potential obtained for the average analyses and their respective standard deviation of the micelles developed.

Table 2. Vesicle diameter of equivalent nanocapsules over the 30 days of study.

Method	PhysicaL-Chemical Parameters	LP_{BR}	LP_{EP}
Laser diffraction	(μm)	220 ± 0.57	287 ± 0.86
	SPAN	1.71 ± 2.41	2.04 ± 0.00
Photon correlation spectroscopy	Z-average (nm)	232 ± 0.00	260 ± 1.70
	PDI	0.210 ± 0.00	0.210 ± 0.01
Electrophoretic mobility	ζ potential (mV)	−16.9 ± 0.37	−20.07 ± 1.10
pH	pH	7.28 ± 0.03	7.35 ± 0.05

Results are presented as the mean ± standard deviation from three batches of the formulation.

The average diameter of the vesicles of the LP_{BR} and LP_{EP} formulations, by laser diffraction, was below 300 nm, presenting a monomodal system, without the presence of nanometric-sized microparticles. Polydispersity demonstrated low variation, with SPAN values around 2.0. The average size of the vesicles by photon correlation spectroscopy corroborates the nanometric diameter already found by laser diffraction. The polydispersity indices (PDI) were close to 0.210, indicating low polydispersity, which makes the colloidal system stable. Zeta potential (ζ) analyses demonstrated values around 20 mv, in modulus, indicating the steric stabilization method, as expected for these nanosystems.

The non-ionic surfactants in the formulations perform steric stabilization, causing a reduction in zeta potential [49]. The pH values of the formulations were close to neutrality, an expected result since a buffer solution (pH 7.4) was used as a vehicle, in addition to the cationic group in the formulation, resulting from phosphatidylcholine, which also provided a neutral pH to the liposomes [50,51]. The analytical method developed in HPLC-UV was validated and shown to be linear, precise, accurate, and robust. The content of the quercetrin compound present in the extract was 98.8 ± 0.9%, with a retention time of 34.0 min.

3.2. Food Consumption of Animals from the First to Eighth Week and Tissue Weight

A significant difference was observed in the final weight of the animals submitted to the high-calorie diet, and a significant increase was seen in the average weight gain of O and OP groups compared to the C group. The statistical results revealed disparities in food intake between the groups submitted to the hypercaloric diet (O and OP), with a notable reduction in feed intake and a corresponding increase in water intake in these groups. Additionally, a progressive increase in caloric intake was observed throughout the study period in the groups submitted to a high-calorie diet, and treatment with the *P. heptaphyllum* liposome did not reverse this parameter (Table 3).

Table 3. Anthropometric parameters and food consumption.

Parameters	Control (C)	Protium (P)	Obese (O)	Obese + Protium (OP)
Initial body weight (g)	167.00 ± 33.80	185.40 ± 48.66	175.40 ± 38.87	167.60 ± 36.10
Final body weight (g)	346.30 ± 28.36	344.20 ± 42.65	428.70 ± 37.39 *	443.40 ± 44.30 *
Weight gain (g)	12.13 ± 5.46	15.00 ± 2.77	33.13 ± 6.46 *	32.50 ± 8.10 *
Food intake (g/day/rat)	23.35 ± 1.58	23.95 ± 1.63	17.73 ± 2.05 *	17.88 ± 1.74 *
Water intake (mL/day/rat)	36.61 ± 2.28	39.29 ± 2.74	54.89 ± 7.77 *	52.74 ± 7.27 *
Calorie intake (kcal/day/rat)	88.06 ± 5.97	90.32 ± 6.17	157.03 ± 11.12 *	155.20 ± 13.69 *
Epididymal adipose tissue (g)	4.30 ± 0.84	4.81 ± 0.96	8.98 ± 1.27 *	13.24 ± 1.92 *$^{/\#}$
Retroperitoneal adipose tissue (g)	5.75 ± 1.37	6.05 ± 2.17	12.88 ± 1.64 *	18.15 ± 2.19 *$^{/\#}$
Liver (g)	10.68 ± 1.00	11.01 ± 1.76	13.23 ± 0.62 *	13.59 ± 1.58 *
HSI (hepatosomatic index) (%)	3.13 ± 0.11	3.26 ± 0.39	2.90 ± 0.34	3.11 ± 0.12

Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O.

Regarding tissue weight, there was an increase in epididymal and retroperitoneal adipose tissue in O and OP groups compared to the C group. Treatment with the liposome increased this same adipose tissue when comparing obese animals. There was an increase in liver weight in animals belonging to these same groups (O and OP group compared to C group), but the hepatosomatic index did not change in the groups analyzed (Table 3).

There was an increase in the average body weight of the O and OP groups from the sixth week until the end of treatment in the eighth week, and the *P. heptaphyllum* liposome was unable to reduce this increase (Figure 1).

There was no statistical difference between the groups concerning IPITT (Figure 2A,B), demonstrating that there was no change in insulin sensitivity in animals treated or not treated with *P. heptaphylllum*. Regarding the OGTT, no statistical differences were observed in the glycemic curve (Figure 2C), but we found that the OP group showed greater glucose intolerance, which was evidenced by the area under the curve (Figure 2D) when compared to the C and O groups.

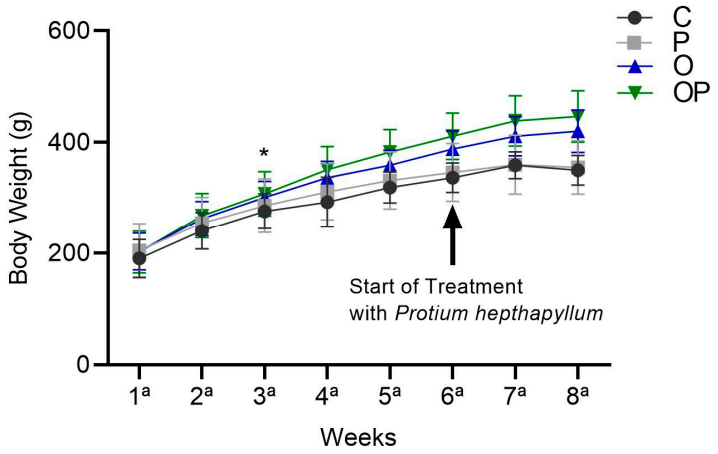

Figure 1. Weight evolution of animals in the Control (C), Protium (P), Obese (O), and Obese Protium (OP) groups between the 1st and 8th week of the experimental protocol (n = 8 animals per group). Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. * $p < 0.05$ vs. C.

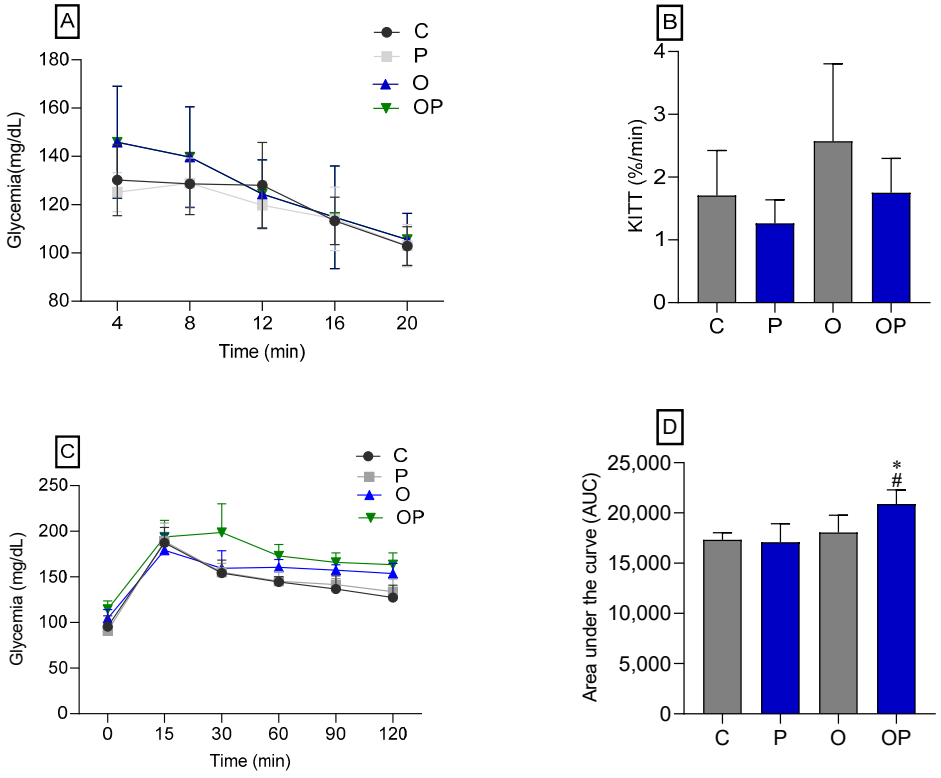

Figure 2. Glycemic curve (**A**) and glucose decay constant (KITT) (**B**), both obtained through the Intraperitoneal Insulin Tolerance Test (IPITT) of the Control (C), Protium (P), Obese (O), and Obese Protium (OP) groups. Glycemic curve (**C**) and area under the curve (AUC) (**D**), obtained through the Oral Glucose Tolerance Test (OGTT) of the Control (C), Protium (P), Obese (O), and Obese Protium (OP) groups (n = 8 per experimental group). Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O.

3.3. Analysis of Plasma Biochemical Parameters

The high-calorie diet increased glucose levels in the O and OP groups when compared to the C group (Table 4), and the *P. heptaphyllum* extract was not able to reverse the animals' blood glucose levels within normal limits compared to reference values. To evaluate liver functions, serum activities of the enzymes alanine aminotransferase (ALT), aspartate

aminotransferase (AST), and alkaline phosphatase (ALP) were measured. The enzymes ALT, AST, and ALP decreased significantly in the O and OP groups compared to the C group, and the P group also showed a decrease in the AST activity when compared to the C group (Table 4). Furthermore, for creatinine, there was a reduction in the P, O, and OP groups when compared to group C. On the other hand, the parameters of total proteins, amylase, and lipase did not demonstrate statistical differences between the groups studied, as detailed in Table 4.

Table 4. Plasma biochemical parameters in the treated groups.

Parameters	Control (C)	Protium (P)	Obese (O)	Obese + Protium (OP)
Glucose (mg/dL)	114.40 ± 29.59	136.00 ± 14.86	184.25 ± 9.19 *	177.37 ± 23.31 *
Total proteins (mg/dL)	6.73 ± 0.81	5.56 ± 1.02	5.92 ± 1.20	7.12 ± 0.74
ALT (U/L)	75.50 ± 14.02	62.00 ± 11.22	45.88 ± 6.81 *	40.88 ± 7.84 *
AST (U/L)	48.47 ± 12.68	30.53 ± 7.50 *	29.53 ± 10.81 *	26.88 ± 8.02 *
ALP (U/L)	179.10 ± 26.97	168.80 ± 24.74	115.90 ± 16.106 *	100.40 ± 10.73 *
Creatinine (mg/dL)	1.30 ± 0.11	0.96 ± 0.09 *	0.85 ± 0.17 *	0.71 ± 0.23 *
Amylase (U/L)	623.80 ± 117.30	644.50 ± 78.89	680.40 ± 61.02	713.20 ± 58.05
Lipase (U/L)	25.00 ± 5.90	19.75 ± 2.65	21.63 ± 6.18	25.88 ± 3.52

Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. * $p < 0.05$ vs. C. (ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase).

Among the parameters related to the lipid profile, we observed a reduction in LDL cholesterol levels in the P and OP groups compared to the C group, as well as a reduction in VLDL levels in the P, O, and OP groups compared to the C group. However, for triglycerides, there was an increase in the OP group compared to the O group (Table 5). The TG/HDL ratio was increased in the OP group compared to the C and O groups. Regarding total cholesterol and HDL cholesterol, no significant changes attributed to the extract were observed in this 14-day experimental protocol.

Table 5. Plasma biochemical parameters regarding lipid profile.

Parameters	Control (C)	Protium (P)	Obese (O)	Obese + Protium (OP)
Cholesterol (mg/dL)	149.60 ± 17.20	130.00 ± 19.49	135.90 ± 20.67	128.40 ± 14.97
HDL (mg/dL)	44.38 ± 2.82	45.88 ± 6.70	39.63 ± 3.37	39.75 ± 4.95
LDL (mg/dL)	88.35 ± 10.70	73.46 ± 10.07 *	78.28 ± 11.76	73.10 ± 12.11 *
VLDL (mg/dL)	21.43 ± 6.26	13.78 ± 3.18 *	13.20 ± 3.09 *	15.88 ± 2.44 *
Triglycerides (mg/dL)	81.04 ± 9.11	68.88 ± 15.91	72.25 ± 7.24	90.50 ± 10.52 #
TG/HDL (mg/dL)	1.82 ± 0.14	1.50 ± 0.25	1.83 ± 0.25	2.31 ± 0.41 */#

Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O. (HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; TG/HDL: triglycerides/high-density lipoprotein ratio).

3.4. Adipose Tissue

3.4.1. Analysis of Parameters of Oxidative and Metabolic Stress in Adipose Tissue

A reduction in SOD enzyme activity was observed in the OP group compared to the O group. There were no significant changes between groups for CAT, GST, GSH, and ASA. On the other hand, TBARS levels increased in the O and OP groups compared to the C group, and carbonyl levels were reduced in the P group compared to the C group (Table 6).

Table 6. Oxidative stress parameters analyzed in the adipose tissue of rats submitted to a high-calorie diet in the experimental groups.

Parameters	Control (C)	Protium (P)	Obese (O)	Obese + Protium (OP)
SOD (IU SOD/mg protein)	23.94 ± 3.02	22.58 ± 4.18	27.84 ± 5.95	20.80 ± 2.76 #
CAT (µmol/min/mg protein)	7.30 ± 3.06	5.53 ± 2.28	6.37 ± 2.14	6.00 ± 2.26
GST (µmol GS-DNB/min/mg protein)	77.53 ± 27.06	74.75 ± 34.99	105.40 ± 38.99	78.25 ± 20.63
GSH (µmol GSH/mg protein)	32.91 ± 12.32	50.68 ± 16.42	30.58 ± 12.74	40.94 ± 16.30
ASA (µmol ASA/g tissue)	0.36 ± 0.08	0.33 ± 0.10	0.33 ± 0.06	0.30 ± 0.05
TBARS (nmol MDA/mg protein)	0.60 ± 0.18	0.65 ± 0.29	1.46 ± 0.38 *	1.96 ± 0.59 *
Carbonyl (nmol carbonyl/mg protein)	254.10; 254.90	129.40; 124.98 *	167.90; 141.26	162.80; 116.93
Glucose (µmol/g tissue)	5.29 ± 1.69	2.81 ± 1.96	4.07 ± 2.03	3.71 ± 2.81
Glycogen (µmol/g tissue)	11.85 ± 2.52	8.14 ± 1.86 *	5.04 ± 2.71 *	3.94 ± 0.48 *
Amino acids (mmol/g tissue)	0.0026 ± 0.0009	0.0025 ± 0.0004	0.0018 ± 0.0006	0.0016 ± 0.0004 *
Ammonia (µmol/ammonia g tissue)	0.48 ± 0.26	1.30 ± 0.18 *	1.23 ± 0.11 *	0.36 ± 0.14 #
Lactate (µmol/g tissue)	1.81 ± 0.63	3.17 ± 0.45 *	1.66 ± 0.40	2.03 ± 0.25
Total proteins (mg/mL)	0.46 ± 0.22	0.78 ± 0.37	0.62 ± 0.35	0.58 ± 0.11

Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. For carbonyl analysis, the Kruskall–Walli's test followed by Dunn's post-hoc test was used, and values were expressed as median and total range. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O. (SOD: superoxide dismutase; GST: glutathione-S-transferase; ASA: ascorbic acid; TBARS: substances reactive to thiobarbituric acid; carbonyl: carbonylated proteins).

Regarding metabolites, both glucose and total proteins did not reveal statistically significant differences between the groups. However, there was a reduction in glycogen levels in the P, O, and OP groups compared to the C group. Amino acids were reduced in the OP group compared to the C group. There was an increase in ammonia levels in groups P and O compared to group C. On the other hand, there was a reduction in these levels in the OP group when compared to the O group. As for lactate, an increase in levels was observed in the P group compared to the C group (Table 6).

3.4.2. Analysis of Cytokines in Adipose Tissue

No statistically significant differences were identified between the groups in TNF-α, IFN-γ, IL-10, IL-17, and IL-1β (Figure 3A,B,D–F, respectively). However, regarding IL-6, a reduction in this cytokine was found in O and OP tissues when compared to group C (Figure 3C).

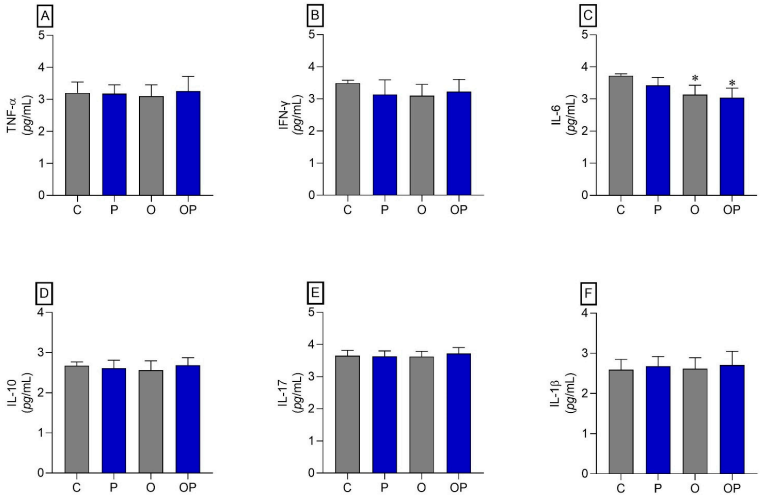

Figure 3. Adipose tissue cytokines. (**A**) TNF-α: Alpha Tumor Necrosis Factor, (**B**) IFN-γ: interferon gamma, (**C**) IL-6: interleukin 6 cytokines, (**D**) IL-10: interleukin 10, (**E**) IL-17: interleukin 17, and (**F**) IL-1β: interleukin 1β; $n = 8$ per experimental group. Value represents mean ± standard deviation. * $p < 0.05$ vs. C. For statistical analysis, data were log-transformed.

3.5. Liver Tissue

3.5.1. Analysis of Parameters of Oxidative and Metabolic Stress in Liver Tissue

No changes were observed in the groups analyzed concerning the parameters of SOD, CAT, GST, GPx, GSH, ASA, and carbonyl; however, there was an increase in TBARS in the P and O groups when compared to the C group (Table 7).

Table 7. Oxidative stress parameters analyzed in the liver tissue of rats submitted to a high-calorie diet in the experimental groups.

Parameters	Control (C)	Protium (P)	Obese (O)	Obese + Protium (OP)
SOD (IU SOD/mg protein)	11.06 ± 1.03	12.08 ± 3.85	10.46 ± 2.78	10.50 ± 2.65
Catalase (µmol/min/mg protein)	8.48 ± 1.96	9.42 ± 3.63	9.23 ± 3.01	12.01 ± 1.34
GST (µmol GS-DNB/min/mg protein)	250.10 ± 48.60	298.00 ± 76.00	241.50 ± 74.10	227.50 ± 84.10
GPx (µmol/min/mg protein)	31.60 ± 9.00	39.30 ± 12.30	23.50 ± 7.40	23.40 ± 5.90
GSH (µmol GSH/mg protein)	20.94 ± 5.82	20.26 ± 4.57	21.45 ± 6.73	22.53 ± 7.37
ASA (µmol ASA/g tissue)	3.16 ± 0.65	2.96 ± 0.52	2.81 ± 0.44	3.04 ± 0.44
TBARS (nmol MDA/mg protein)	0.14 ± 0.02	0.22 ± 0.03 *	0.23 ± 0.03 *	0.19 ± 0.04
Carbonyl (nmol carbonyl/mg protein)	15.85 ± 1.04	12.40 ± 2.45	13.31 ± 3.86	14.67 ± 2.63
Glucose (µmol/g tissue)	31.27 ± 10.79	66.01 ± 18.55 *	90.81 ± 25.10 *	62.42 ± 16.62 */#
Glycogen (µmol/g tissue)	1.53 ± 0.24	1.96 ± 0.28 *	1.63 ± 0.32	1.84 ± 0.37
Amino acids (mmol/g tissue)	0.10 ± 0.02	0.08 ± 0.01 *	0.07 ± 0.009 *	0.11 ± 0.01 #
Ammonia (µmol ammonia/g tissue)	1.09 ± 0.33	0.60 ± 0.19 *	0.81 ± 0.27	0.75 ± 0.26
Lactate (µmol/g tissue)	1.66; 1.23	1.82; 1.29	0.85; 0.95 *	1.53; 1.25 #
Total proteins (mg/mL)	6.65 ± 0.21	7.02 ± 2.13	6.58 ± 1.66	7.25 ± 2.46

Results are presented as the mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test. For lactate analysis, the Kruskall–Wallis test followed by Dunn's post-hoc test was used, and the values are expressed as the median and total range. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O. (SOD: superoxide dismutase; GST: glutathione-S-transferase; GPx: glutathione peroxidase; ASA: ascorbic acid; TBARS: substances reactive to thiobarbituric acid; carbonyl: carbonylated proteins).

Considering the levels of metabolites in the liver tissue, there was an increase in glucose concentration in the P, O, and OP groups compared to the C group. However, *P. heptaphyllum* was able to correct the levels in the OP group when compared to the O group. Glycogen concentration showed an increase in the P group compared to the C group. For amino acid levels, there was a decrease in the P and O groups compared to the C group, and *P. heptaphyllum* corrected these amino acid levels in the OP group when compared to the O group. The ammonia concentration showed a decrease in group P compared to group C, and lactate levels showed a decrease in group O compared to the group C; animals treated with extract liposomes (OP) recovered these values to control levels. Total proteins showed no statistical difference between the groups studied.

3.5.2. Analysis of Cytokines in Liver Tissue

The results indicated that there was an increase in IFN-γ in the O and OP groups compared to the C group (Figure 4B). The cytokine IL-10 also showed an increase in the P and O groups when compared to the C group (Figure 4D). On the other hand, the cytokine IL-1β increased in group O compared to group C, and plant liposomes were effective in reducing the levels of this cytokine in group OP (Figure 4F). The cytokines TNF-α, IL-6, and IL-17 did not show statistically significant differences (Figure 4A,C,E, respectively).

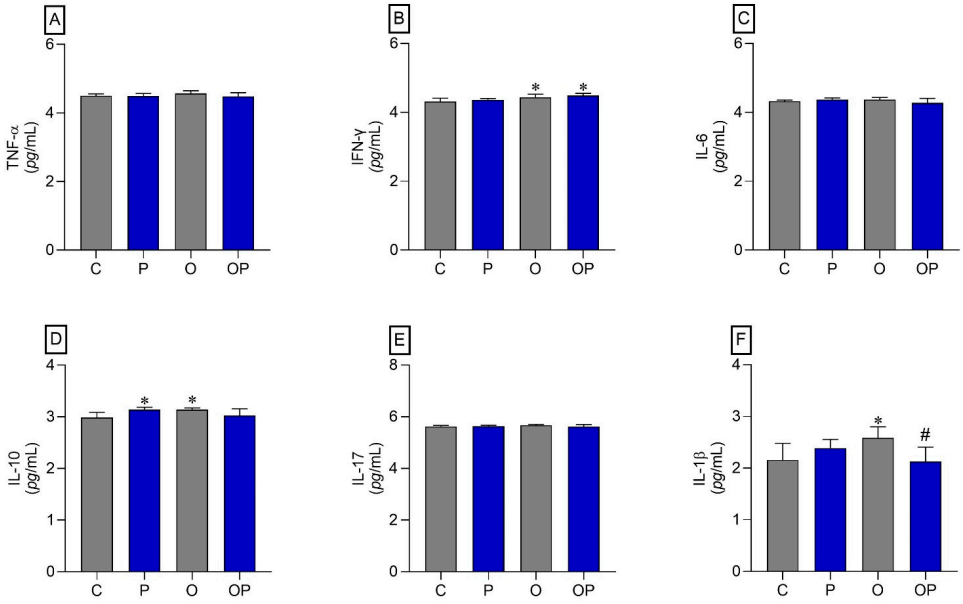

Figure 4. Liver tissue cytokines. (**A**) TNF-α: Alpha Tumor Necrosis Factor, (**B**) IFN-γ: interferon gamma, (**C**) IL-6: interleukin 6, (**D**) IL-10: interleukin 10, (**E**) IL-17: interleukin 17, and (**F**) IL-1β: interleukin 1β; $n = 8$ per experimental group. Value represents mean ± standard deviation. * $p < 0.05$ vs. C; # $p < 0.05$ vs. O. For statistical analysis, data were log transformed.

3.5.3. Histopathological Analysis of Liver Tissue

The liver of animals fed a standard diet (C and P) showed well-formed nucleated hepatocytes, well-formed sinusoidal capillaries between hepatocyte plates, absence of inflammatory infiltrate, fibrous tissue, or lipid accumulation, and intact architecture compatible with a normal liver (Figure 5—C and P). The groups that received a high-calorie diet (O) and were treated with *P. heptaphyllum* liposomes (OP) showed hepatic steatosis with vesicular-appearing cytoplasm, with many fat droplets of various sizes scattered around (Figure 5—O and OP).

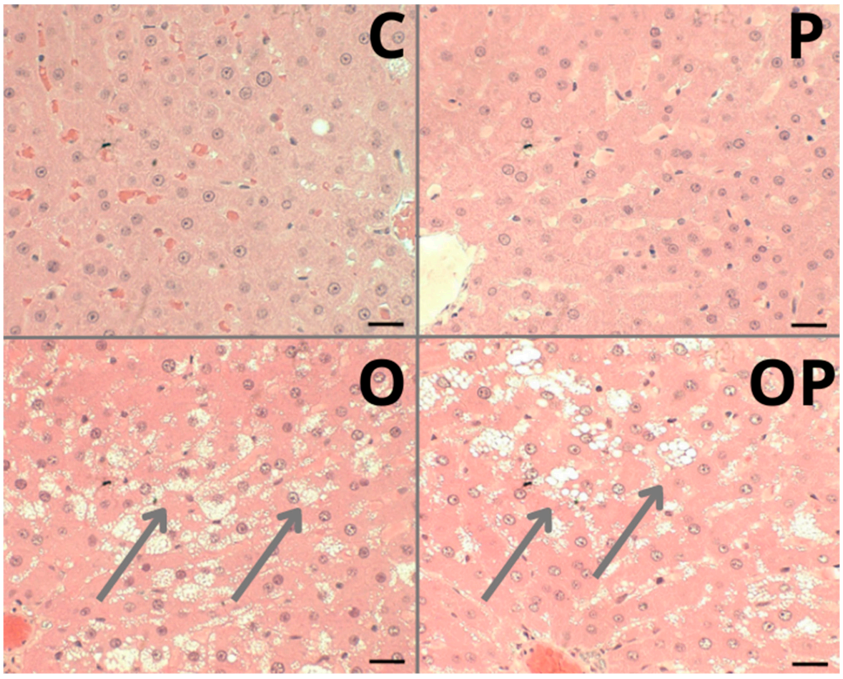

Figure 5. Photomicrograph of a sample from an individual from each liver tissue group showing different morphological changes in the Control (C), Protium (P), Obese (O) and Obese Protium (OP) groups. Steatosis: (arrows) groups O and OP. Resolution: Bar 30 μm.

4. Discussion

We used nanotechnology to create liposomes from *P. heptaphyllum* extract, with the aim of making the product more effective and reducing possible adverse effects [13]. This approach improves the absorption of bioactive substances, such as flavonoids present in the extract, especially when administered orally [51]. The use of nanocarriers has become a common approach to reduce toxic effects and enhance active ingredient activity [52]. Liposomes were prepared by the method of lipid film hydration followed by extrusion [30]. This method is widely used due to its reproducibility and easy handling, resulting in an opalescent liquid that can be used directly after preparation. The liposomes showed a bluish white color due to the Tyndall effect, characteristic of concentrated colloidal solutions. LP_{EP} had an opalescent milky green color, with a slight odor characteristic of the extract used. All formulations were macroscopically homogeneous with a bluish reflection, resulting from the Brownian movement of the nanomicelles. The techniques used to evaluate the diameters of the formulations showed the presence of nanometric micelles, without any microscopic samples. Furthermore, the low polydispersity values demonstrated narrow size distributions and uniformity in mean diameters for all formulations developed. The pH neutrality is consistent with the composition of the nanosystems in this study. All these parameters qualified the LP_{EP} and LP_{BR} formulations for application in biological models.

The induced obesity model used in the present study was confirmed by the increase in average body weight and the accumulation of body fat, reflected in the increase in organ weight. Furthermore, comprehensive data such as adipose tissue accumulation and hyperglycemia, typical features of obesity and associated metabolic disorders, corroborate the effectiveness of the model. These findings are consistent with other studies that used obesity induction. For example, previous studies reported the induction of obesity in rats on a high-fat diet [53]. Similarly, a high-fat and high-protein diet, commonly used for weight loss, induced obesity in rats [54], and another study used an obesity induction protocol and treated rats with botryosphaeran [(1→3) (1→6)-β-d-glucan], obtaining beneficial metabolic, antioxidant, and anti-inflammatory effects [55]. Finally, it is claimed that high-fat diets are effective in modeling the metabolic disorders of human obesity in rodents [56]. Although the study protocol [54] is like ours, we were unable to observe positive changes in liposomes in the epididymal and retroperitoneal adipose tissue depots, since the results were greater in the OP group compared to the O group. This unexpected result may be attributed to the insufficient dose of the extract and the 14-day treatment period. The liposomal form of the extract may also have interfered with the bioavailability of the bioactive compounds. Studies show that obesity is associated with oxidative stress, and treatments with natural extracts can be toxic and should be used with caution, as they can cause adverse effects in the long term or at incorrect doses. Furthermore, the complexity of the herbal composition makes it difficult to determine the mechanisms of action, as discussed by studies that provide an overview of the scientific evidence on the use of herbal medicines in the treatment of obesity [57].

Animals that received a high-calorie diet (O) and were treated with liposomes (OP) showed a significantly larger area under the curve in the OGTT, suggesting signs of glucose intolerance. Hypercaloric diets induce metabolic disorders like human metabolic syndrome [56,58] and, because of obesity triggered by the high caloric value of the diet, changes in glycemia [59]. However, in this study, the obese groups (O and OP) presented high plasma glycemia, without reversal by treatment with *P. heptaphyllum,* but previous studies reveal the action of the resin of *P. heptaphyllum* in reducing plasma glucose in the face of obesity induced by a high-fat diet in rats [60]. Obesity is associated with increased liver enzymes, markers of liver function, with hepatic steatosis being common in obese individuals, which can cause liver damage and result in the release of these enzymes into the bloodstream [61,62]. The reduction observed in the activities of the hepatic enzymes ALT, AST, and ALP in the obese groups (O and OP) demonstrates that the model developed did not cause damage to the point of increasing these activities, nor did treatment with liposomes containing *P. heptaphyllum* interfere with these parameters. Previous studies

showed that *P. heptaphyllum* resin attenuated the acute paracetamol-induced increase in serum ALT and AST activities in mice [19]. Accordingly, the activity of ALT, AST, and ALP enzymes increased in the plasma of mice exposed to paracetamol, but ethyl acetate extract of *P. heptaphyllum* decreased these activities by controlling ALT and AST activities [28]. Studies in rats on a high-fat diet for 15 weeks showed that *P. heptaphyllum* also triggered a decrease in liver enzyme activities [62]. Furthermore, we observed that the treated groups also showed a decrease in creatinine levels, in line with similar results in rats on a high-calorie diet and lycopene treatment [63]. We can observe that previous studies showed good results with *P. heptaphyllum*, and our results, despite not showing many advances in these parameters, may have been influenced by the treatment time and the dose of the extract. Added to that, the innovative methodology of liposomes presents challenges in understanding the bioactives involved.

Regarding the lipid profile, the P and OP groups showed a decrease in LDL and VLDL levels, which can be attributed to the flavonoids present in *P. heptaphyllum*, such as quercetin-3-β-D-glucoside, myricetin, and quercetrin. Studies have discussed the antioxidant potential and other biological activities of *P. heptaphyllum*, highlighting these bioactive compounds [64]. Furthermore, they demonstrated that quercetrin can reduce cholesterol and prevent atherosclerosis [65], reporting the lipid-lowering effects of the hydroalcoholic extract of *Solidago chilensis* and its main isolated constituent in cholesterol-fed rats, demonstrating the ability of quercetrin to reduce cholesterol and prevent atherosclerosis.

In addition, the triterpenes α- and β-amyrin present in *P. heptaphyllum* resin also reduce LDL and VLDL lipoproteins, such as in a study that investigated the antihyperglycemic and lipid-lowering effects of the mixture of triterpenes α- and β-amyrin from *P. heptaphyllum* in mice, showing a reduction in LDL and VLDL lipoproteins [66]. Another study investigated the bioactive triterpenes from *P. heptaphyllum* resin extract, noting their cholesterol-lowering potential, especially in LDL lipoproteins [67]. The hypercaloric diet applied was rich in lipids and not in carbohydrates, which explains the low production of hepatic TG and reduction in VLDL and LDL. However, an increase in triglycerides and the TG/HDL ratio was observed in the treated group (OP), associated with an increase in adipose tissue, crucial for triglyceride storage, since the fate of excess fat absorbed from the diet is delivered directly to adipose tissue for storage via chylomicrons [68]. Research has reported increased visceral and subcutaneous adipose tissue in children with acute pancreatitis, highlighting the importance of adipose tissue in triglyceride storage [69]. In addition, the relationship between the triglycerides/HDL index (TG/HDL) as a risk marker for metabolic syndrome and cardiovascular diseases was discussed, correlating it with the increase in adipose tissue and lipid profile [70]. The presence of the plant during this treatment period did not affect this marker. This link between high-fat diets and metabolic changes highlights the potential adverse effects of these diets on plasma and liver lipid profiles. Research has examined the effects of high-calorie diets on glucose homeostasis in rats, highlighting the influence of saturated and monounsaturated dietary lipids on plasma and liver lipid profiles [68].

Adipose tissue, a vital component of the human body, plays a fundamental role in metabolic and homeostatic functions. In the present study, an increase in TBARS levels in the adipose tissue of obese groups (O and OP) was observed, indicating greater oxidative stress and possible cellular damage in this tissue. On the other hand, other studies have already demonstrated positive effects of *P. heptaphyllum* in reducing TBARS and carbonyl. For instance, the investigation of the anti-inflammatory effect of the triterpenes α- and β-amyrin from *P. heptaphyllum* in a model of acute periodontitis in rats showed a significant reduction in oxidative stress, especially TBARS [23]. Another study [28] observed carbonyl reduction in kidney tissue samples from mice exposed to paracetamol and treated with the ethyl acetate fraction of *P. heptaphyllum*. On the other hand, the activity of the SOD enzyme decreased in the OP group when compared to the O group. Several studies demonstrate the antioxidant potential of flavonoids, especially quercetin, which acts directly on antioxidant enzymes. In this context, the antioxidant capacities of flavonoids present in cherries (*Prunus pseudocerasus*) were evaluated, including astragalin, cyanidin-3-O-glucoside, cinaroside,

quercetin, rutin, and vitexin, and treatments with high doses of cyanidin-3-O-glucoside and rutin increased SOD activities in the serum, liver, kidney, and heart of mice, while reducing the level of MDA (malondialdehyde) in these tissues [71]. Quercetin, a bioactive flavonoid with several antioxidant properties, has been highlighted for its biological importance and its role in protecting against oxidative stress [72]. Additionally, studies analyzing the antioxidant activities of quercetin and its complexes have observed significant beneficial effects for medicinal applications, particularly in reducing oxidative damage [73]. However, in this study, we did not observe a pattern of protection of liposomes containing the extract in this tissue against enzymatic and non-enzymatic activities or inflammatory markers, even after a 14-day treatment. It is possible that a longer treatment time will be required.

This research is pioneering with *P. heptaphyllum* extract liposomes, including redox and metabolic analyses, demonstrating an innovative approach. The reduction in glycogen in groups O and OP in adipose tissue suggests changes in energy metabolism associated with insulin resistance in obesity, indicating a possible reduction in glucose storage capacity [74]. The decrease in amino acids and ammonia in the OP group reflects specific metabolic adjustments, indicating a positive response to treatment [75]. Obesity can affect amino acid metabolism, resulting in changes in the production and excretion of ammonia, a metabolic byproduct whose obesity-related imbalances can lead to elevated levels of this compound [76].

When natural liver protective mechanisms fail, liver damage can occur. Thus, treatment with liposomes for 14 days in obese animals did not promote changes in enzymatic antioxidants (SOD, CAT, GST, and GPx), but the TBARS marker, a sign of lipid damage, remained elevated (groups P and O) in liver tissue, without causing protection against obesity, and both triggered lipoperoxidation during this treatment period. Previous studies from our group highlighted the ability of *P. heptaphyllum* extracts to reduce hepatic oxidative stress in a paracetamol-induced liver damage model [28]. It is known that quercitrin present in *P. heptaphyllum* extract is recognized for its bioactivity, and it has been studied in various health conditions, exhibiting antioxidant, anti-inflammatory, and antimicrobial properties [77]. Furthermore, some authors [78] have suggested that flavonoids, including quercetin, directly impact mitochondrial processes, indicating potential to counteract complications associated with obesity. However, even though the liposomes under study contain these flavonoids, it is important to explain that we carried out a 14-day treatment, with a different form of dispensing, and it is possible that these conditions were not ideal for the possible benefits in this organ under obesity induction.

In the obese group treated with *P. heptaphyllum* (OP), there was a reduction in glucose levels compared to the obese group, indicating a possible benefit of the treatment on liver tissue. *P. heptaphyllum* restored amino acid and lactate levels in the OP group, suggesting modulation of these parameters attributable to flavonoids such as quercetrin present in the plant. Studies [79] have indicated that high concentrations of plasma glucose and lactate result in greater uptake in peripheral tissues, regardless of the type of diet. Furthermore, the liposome per se promoted an increase in glucose and hepatic glycogen stores combined with a reduction in amino acids and ammonia. The liver is responsible for carrying out the urea cycle, an important route for ammonia excretion. It is possible that the amino acids provided their carbon skeletons to produce glycogen, and the ammonia generated was efficiently destined for its elimination via the urea cycle [70].

Flavonoids such as quercetin are recognized for anti-inflammatory and cytokine immunomodulatory properties. In the liver tissue, there was an increase in IFN-γ (groups O and OP), IL-10 (groups P and O), and IL-1β (group O) compared to the control, showing an inflammatory process mediated by T helper lymphocytes 1 (Th1) and inflammasome activation in the obese group. The increase in IL-10 in obese individuals may be due to an attempt to control the inflammatory process in this tissue. The use of liposomes in obese animals managed to reduce IL-1β levels in the liver, probably reducing the NLRP3 (NOD-like receptor family pyrin domain-containing 3) and inflammasome activation pathway. The anti-inflammatory effect related to *P. heptaphyllum* was reported in an animal model of periodontitis, where the plant isolates, α- and β-amyrin, were administered 2 h before the induction of periodontitis.

After 6 h, there was a decrease in TNF-α levels in treated animals, although other cytokines were not measured [23]. Previous studies [80] also highlighted the ability to reduce inflammatory cytokines in inflammation models where the effects of quercetin-loaded liposomes were investigated in a mouse model of sepsis, demonstrating that liposomal encapsulation promoted the inhibitory effects of quercetin on lung-mediated inflammation by macrophages, reducing mortality without apparent toxicity.

Histological evaluation revealed hepatic steatosis in groups O and OP. Unfortunately, treatment with liposomes failed to prevent this change caused by obesity, contradicting the findings of other studies that also investigated the same plant. However, using other components present in it, such as the triterpenes α- and β-amyrin extracted from the trunk resin, conferred significant protection against acetaminophen-induced liver injury in mice, preventing hepatic congestion and centrilobular necrosis [19]. In addition, other works with *P. heptaphyllum* resin showed that it prevents microgoticular steatosis and liver inflammation in mice fed a high-fat diet, preserving normal liver morphology [60], and administration of α- and β-amyrin in streptozotocin-treated diabetic mice protects the islets of Langerhans from cellular destruction, maintaining the morphological integrity of the pancreas, like normal controls [66]. Additionally, studies with the same resins from *P. heptaphyllum* showed prevention of inflammatory infiltrations and the accumulation of lipid droplets in the liver of mice fed a high-fat diet, preserving normal liver morphology [25].

In short, the results showed that the use of liposomes containing *P. heptaphyllum* extract for 14 days showed an improvement in the functional and inflammatory parameters of the liver in obese animals. However, the suggested treatment was not effective in alleviating general changes related to obesity, such as weight gain, fat, glucose, triglycerides, and inflammation in adipose tissue.

5. Final Considerations

The use of nanotechnology to develop liposomes with *P. heptaphyllum* extract sought to improve the effectiveness of the treatment and minimize potential adverse effects. Physicochemical analyses confirmed the quality of the formulations, qualifying the liposomes for application in biological models. In the context of induced obesity in rats, liposomes containing extract, although showing significant benefits in the liver, were not effective in reversing the metabolic, histological, and inflammatory changes associated with obesity. A positive response to treatment was observed, evidenced by the reduction in oxidative stress markers, restoration of metabolic parameters in the liver, and modulation of inflammatory cytokines. However, the treatment did not influence weight gain, glucose levels, triglycerides, or inflammation in adipose tissue, indicating limitations in reversing systemic changes related to obesity. Thus, although promising, liposomes with *P. heptaphyllum* extract proved to be only partially effective given the complexity of obesity manifestations, highlighting the need for more in-depth investigations and complementary strategies.

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Institutional Review Board Statement: The animal study protocol was approved by the Guidelines of the Ethics Committee on the Use of Animals of the Federal University of Mato Grosso no. 23108.031684/2021-21.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

1. Ayogu, R.N.B.; Oshomegie, H.; Udenta, E.A. Energy intake, expenditure and balance, and factors associated with energy balance of young adults (20–39 years): A retrospective cross-sectional community-based cohort study. *BMC Nutr.* **2022**, *8*, 142. [CrossRef]
2. Sousa, M.E.d.S.; Alves, S.A.S.; Sousa, M.T.d.S.L.; Silva, B.H.M.; Lima, D.S.; Lima, I.S.; Macedo, I.D.S.; Barbosa, A.K.d.S. Food and herbal medicine as a way to prevent childhood obesity. *Res. Soc. Dev.* **2021**, *10*, e356101018939. [CrossRef]
3. Chowbey, P.; Narwaria, M.; Kumeran, R.; Dukkipati, N.; Todkar, J.A. A Review on Obesity and its Management: Focus on Meal Replacement Therapy. *J. Clin. Diagn. Res.* **2022**, *16*, OE01–OE06. [CrossRef]
4. Meyhöfer, S.; Lautenbach, A.; Serfling, G. Adiposis—Eine behandlungsbedürftige chronische Erkrankung [Obesity—A chronic disease requiring treatment]. *Dtsch. Med. Wochenschr.* **2022**, *147*, 656–662. [CrossRef]
5. Aswathi, P.; Martinez, L.W.; Radha, S. Action of Reactive Oxygen Species in Metabolic Diseases employed as Biomarkers of Oxidative Stress. *Res. J. Biotech.* **2022**, *17*, 207–215. [CrossRef]
6. Manna, P.; Jain, S.K. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab. Syndr. Relat. Disord.* **2015**, *13*, 423–444. [CrossRef]
7. Kawai, T.; Autieri, M.V.; Scalia, R. Adipose tissue inflammation and metabolic dysfunction in obesity. *Am. J. Physiol. Cell Physiol.* **2021**, *320*, C375–C391. [CrossRef] [PubMed]
8. Sippel, C.; Bastian, R.; Giovanella, J.; Faccin, C.; Contini, V.; Dal Bosco, S. Inflammatory processes of obesity. *Braz. J. Health Sci. Inflamm. Process. Obes.* **2015**, *12*, 48–56. [CrossRef]
9. Cruz, C.K.S.; Ribeiro, R.C.L.; Oliveira, R.C.B.; Brito, R.G.A.; Rocha, G.M.M.; Silva, M.A.; Oliveira, G.A.L. The use of medicinal plants in the treatment of obesity: An integrative review. *Res. Soc. Dev.* **2020**, *9*, e439997167. [CrossRef]
10. Li, S.; Xu, Y.; Guo, W.; Chen, F.; Zhang, C.; Tan, H.Y.; Wang, N.; Feng, Y. The Impacts of Herbal Medicines and Natural Products on Regulating the Hepatic Lipid Metabolism. *Front. Pharmacol.* **2020**, *11*, 351. [CrossRef]
11. Burdaev, N.I.; Nikolaeva, L.L.; Kosenko, V.V.; Shprakh, Z.S.; Bunyatyan, N.D. Liposomes as drug carriers: Classification, preparation methods and medicinal use. Bulletin of the Scientific Center for Specialized Evaluation of Medicines. *Regul. Res. Drug Eval.* **2023**, *13*, 316–332. [CrossRef]
12. Wang, D.-Y.; van der Mei, H.C.; Ren, Y.; Busscher, H.J.; Shi, L. Lipid-Based Antimicrobial Delivery-Systems for the Treatment of Bacterial Infections. *Front. Chem.* **2020**, *7*, 872. [CrossRef]
13. Zanco, P.; Ferrarini, S.R.; Cruz, L.; Ferreira, L.M.; Bicudo, R.C.; Cavalheiro, L.; Vieira Júnior, G.M.; Sugui, M.M. Improved antimutagenic effect of *Pyrostegia venusta* (Ker Gawl.) Miers nanostructured extract in liposome and polymeric nanoparticle. *Braz. J. Pharm. Sci.* **2022**, *58*, e20234. [CrossRef]
14. de Barros, N.B.; Macedo, S.R.A.; Ferreira, A.S.; Tagliari, M.P.; Zanchi, F.B.; Kayano, A.M.; Nicolete, R. Liposomes containing an ASP49-phospholipase A 2 from Bothrops jararacussu snake venom as experimental therapy against cutaneous leishmaniasis. *Int. Immunopharmacol.* **2016**, *36*, 225–231. [CrossRef]
15. Maddiboyina, B.; Ramaiah Nakkala, R.K.; Roy, H. Perspectives on cutting-edge technologies for lipid-based nanoparticulate drug delivery and their applications. *Chem. Biol. Drug Des.* **2023**, *102*, 377–394. [CrossRef]
16. Oliveira da Silva, L.; Assunção Ferreira, M.R.; Lira Soares, L.A. Nanotechnology Formulations Designed with Herbal Extracts and Their Therapeutic Applications—A Review. *Chem. Biodiver.* **2023**, *20*, e202201241. [CrossRef]
17. Renovato-Núñez, J.; Cobos-Puc, L.E.; Viveros-Valdez, E.; Iliná, A.; Segura-Ceniceros, E.P.; Rodríguez-Herrera, R.; Silva-Belmares, S.Y. Nanoencapsulated Systems for Phytopharmaceutical Delivery. In *Advances in New Formulations for Drug Delivery*; Keservani, R.K., Kesharwani, R.K., Sharma, A.K., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2023. [CrossRef]
18. Halevas, E.; Avgoulas, D.; Katsipis, G.; Pantazaki, A. Flavonoid-liposomes formulations: Physico-chemical characteristics, biological activities and therapeutic applications. *Eur. J. Med. Chem. Rep.* **2022**, *5*, 100059. [CrossRef]
19. Oliveira, F.A.; Chaves, M.H.; Almeida, F.R.; Lima, R.C., Jr.; Silva, R.M.; Maia, J.L.; Brito, G.A.; Santos, F.A.; Rao, V.S. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.* **2005**, *98*, 103–108. [CrossRef]
20. Islam, R.; Kabir, M.F.; Alam, R.; Dahr, R.; Rana, M.N.; Islam, E.; Parvin, S.; Hossain, A. Sedative, stability membrane, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall. *Asian Pac. J. Trop. Dis.* **2014**, *4*, 928–933. [CrossRef]
21. Nogueira, A.O.; Oliveira, Y.I.S.; Adjafre, B.L.; de Moraes, M.E.A.; Aragão, G.F. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from *Protium heptaphyllum*: A literature review. *Fundam. Clin. Pharmacol.* **2019**, *33*, 4–12. [CrossRef]
22. Marques, D.D.; Sartori, R.A.; Lemos, T.L.G.; Machado, L.L.; Souza, J.S.N.D.; Monte, F.J.Q. Chemical composition of essential oils from two subspecies of *Protium heptaphyllum*. *Acta Amaz.* **2010**, *40*, 227–230. [CrossRef]

23. Holanda Pinto, S.A.; Pinto, L.M.; Cunha, G.M.; Chaves, M.H.; Santos, F.A.; Rao, V.S. Anti-inflammatory effect of alpha, beta-amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in a rat model of acute periodontitis. *Inflammopharmacology* **2008**, *16*, 48–52. [CrossRef] [PubMed]
24. Aragão, G.F.; Carneiro, L.M.; Junior, A.P.; Vieira, L.C.; Bandeira, P.N.; Lemos, T.L.; Viana, G.S. A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Pharmacol. Biochem. Behav.* **2006**, *85*, 827–834. [CrossRef] [PubMed]
25. Carvalho, K.M.; de Melo, T.S.; de Melo, K.M.; Quinderé, A.L.; de Oliveira, F.T.; Viana, A.F.; Nunes, P.I.; Quetz, J.D.; Viana, D.A.; da Silva, A.A.; et al. Amyrins from *Protium heptaphyllum* Reduce High-Fat Diet-Induced Obesity in Mice via Modulation of Enzymatic, Hormonal and Inflammatory Responses. *Planta Med.* **2017**, *83*, 285–291. [CrossRef] [PubMed]
26. Oliveira, F.A.; Vieira-Júnior, G.M.; Chaves, M.H.; Almeida, F.R.; Florêncio, M.G.; Lima, R.C., Jr.; Silva, R.M.; Santos, F.A.; Rao, V.S. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. *Pharmacol. Res.* **2004**, *49*, 105–111. [CrossRef] [PubMed]
27. Cabral, R.S.C.; Alves, C.C.F.; Batista, H.R.F.; Sousa, W.C.; Abrahão, I.S.; Crotti, A.E.M.; Santiago, M.B.; Martins, C.H.G.; Miranda, M.L.D. Chemical composition of essential oils from different parts of *Protium heptaphyllum* (Aubl.) Marchand and there in vitro antibacterial activity. *Nat. Prod. Res.* **2020**, *34*, 2378–2383. [CrossRef] [PubMed]
28. Patias, N.S.; Sinhorin, V.D.G.; de Moura, F.R.; da Cunha, A.P.F.; Lima, R.R.S.; Costa, R.J.; da Costa, T.B.; Cavalheiro, L.; Bicudo, R.C.; Sinhorin, A.P. Identification of flavonoids by lc-ms/ms in leaves extract from *Protium heptaphyllum* (Aubl.) March and antioxidant activity in mice. *Nat. Prod. J.* **2021**, *11*, 715–727. [CrossRef]
29. Anifowose, S.O.; Alqahtani, W.S.N.; Al-Dahmash, B.A.; Sasse, F.; Jalouli, M.; Aboul-Soud, M.A.M.; Badjah-Hadj-Ahmed, A.Y.; Elnakady, Y.A. Efforts in Bioprospecting Research: A Survey of Novel Anticancer Phytochemicals Reported in the Last Decade. *Molecules* **2022**, *27*, 8307. [CrossRef] [PubMed]
30. Mertins, O.; Sebben, M.; Pohlmann, A.R.; da Silveira, N.P. Production of soybean phosphatidylcholine-chitosan nanovesicles by reverse phase evaporation: A step-by-step study. *Chem. Phys. Lipids.* **2005**, *138*, 29–37. [CrossRef]
31. Malone, M.H. The pharmacological evaluation of natural products-general and specific approaches to screening ethnopharmaceuticals. *J. Ethnopharmacol.* **1983**, *8*, 127–147. [CrossRef]
32. Nascimento, A.F.; Sugizaki, M.M.; Leopoldo, A.S.; Lima-Leopoldo, A.P.; Luvizotto, R.A.; Nogueira, C.R.; Cicogna, A.C. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arq. Bras. Endocrinol. Metab.* **2008**, *52*, 968–974. [CrossRef] [PubMed]
33. Luvizotto, R.A.; Nascimento, A.F.; Imaizumi, E.; Pierine, D.T.; Conde, S.J.; Correa, C.R.; Yeum, K.J.; Ferreira, A.L. Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. *Br. J. Nutr.* **2013**, *110*, 1803–1809. [CrossRef] [PubMed]
34. Nelson, D.P.; Kiesow, L.A. Enthalpy of decomposition of hydrogen peroxide by catalase at 25 degrees C (with molar extinction coefficients of H_2O_2 solutions in the UV). *Anal. Biochem.* **1972**, *49*, 474–478. [CrossRef] [PubMed]
35. Misra, H.P.; Fridovich, I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **1972**, *247*, 3170–3175. [CrossRef] [PubMed]
36. Habig, W.H.; Pabst, M.J.; Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139. [CrossRef] [PubMed]
37. Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158–169. [PubMed]
38. Sedlak, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **1968**, *25*, 192–205. [CrossRef] [PubMed]
39. Roe, J.H. Chemical determination of ascorbic, dehydroascorbic, and diketogulonic acids. *Meth. Biochem. Anal.* **1954**, *1*, 115–139. [CrossRef]
40. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310. [CrossRef]
41. Colombo, G.; Clerici, M.; Garavaglia, M.E.; Giustarini, D.; Rossi, R.; Milzani, A.; Dalle-Donne, I. A step-by-step protocol for assaying protein carbonylation in biological samples. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1019*, 178–190. [CrossRef]
42. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
43. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Roberts, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–358. [CrossRef]
44. Bidinotto, P.M.; Souza, R.H.S.; Moraes, G. Hepatic glycogen in eight tropical freshwater teleost fish: A procedure for field determinations of micro samples. *Tech. Bull.* **1997**, *CEPTA 10*, 53–60. Available online: https://www.icmbio.gov.br/cepta/images/stories/producao_cientifica/hepatic_1997_01.pdf (accessed on 10 June 2024).
45. Harrowe, J.R.; Brown, C.H. Blood lactic acid—A micromethod adapted to field collection of microliter samples. *J. App. Phys.* **1972**, *32*, 709–711. [CrossRef]
46. Spies, J.R. Colorimetric procedures for amino acids. *Methods Enzym.* **1957**, *3*, 467–477. [CrossRef]
47. Gentzkow, C.J.; Masen, J.M. An accurate method for determining blood urea nitrogen by direct nesslerization. *J. Biol. Chem.* **1942**, *143*, 531–544. [CrossRef]

48. da Silva, K.C.; Aguiar, D.H.; Bomfim, G.F. Assessment of the effect of copaiba oil (copaifera sp.) on the kidneys of rats with hepatic dysfunction. *Sci. Electron. Arch.* **2023**, *16*. [CrossRef]
49. Büttner-Pires, J.; Conceição, A.G.B.; Costa, S.A.; Silva, K.P.; Ferrarini, S.R.; Andrade, C.M.B. Evaluation of the cytotoxicity of polymeric nano capsules with a lipid core containing pequi oil (*Caryocar Brasiliense* Cambess) as a structuring agent. *Panor. Mag.* **2020**, *2*, 21–38. Available online: https://periodicoscientificos.ufmt.br/revistapanoramica/index.php/revistapanoramica/article/view/1207/19192391 (accessed on 25 May 2024).
50. Perttu, E.K.; Kohli, A.G., Jr.; Szoka, F.C. Inverse-phosphocholine lipids: A remix of a common phospholipid. *J. Am. Chem. Soc.* **2012**, *134*, 4485–4488. [CrossRef]
51. Dong, X.; Mumper, R.J. Nanomedicinal strategies to treat multidrug-resistant tumors: Current progress. *Nanomedicine* **2010**, *5*, 597–615. [CrossRef]
52. Bilia, A.R.; Piazzini, V.; Guccione, C.; Risaliti, L.; Asprea, M.; Capecchi, G.; Bergonzi, M.C. Improving on Nature: The Role of Nanomedicine in the Development of Clinical Natural Drugs. *Planta Med.* **2017**, *83*, 366–381. [CrossRef] [PubMed]
53. Lim, S.M.; Goh, Y.M.; Mohtarrudin, N.; Loh, S.P. Germinated brown rice improves obesity in high-fat diet induced obese rats. *BMC Complement. Altern. Med.* **2016**, *16*, 140. [CrossRef] [PubMed]
54. Borba, A.J.D.; Rocha, M.G.M.; Silva, M.F.D.; Tibúrcio, D.T.D.S.; Pereira, S.A.D.L.; Reis, L.C.D.; Thedei Júnior, G. High-fat-protein diet used for weight loss induces obesity in rats. *Rev. Nutr.* **2011**, *24*, 519–528. [CrossRef]
55. da Silva, T.P.; Geraldelli, D.; Martins, K.O.; Braga, A.J.L.; Rosa, A.P.; Ferneda, J.M.A.; Bomfim, G.F.; Barbosa-Dekker, A.M.; Dekker, R.F.H.; Dias, M.C.; et al. Antioxidant, anti-inflammatory and beneficial metabolic effects of botryosphaeran [(1→3)(1→6)-β-d-glucan] are responsible for its anti-tumor activity in experimental non-obese and obese rats bearing Walker-256 tumors. *Cell Biochem. Funct.* **2022**, *40*, 213–227. [CrossRef] [PubMed]
56. Buettner, R.; Schölmerich, J.; Bollheimer, L.C. High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity* **2007**, *15*, 798–808. [CrossRef] [PubMed]
57. Liu, Y.; Sun, M.; Yao, H.; Liu, Y.; Gao, R. Herbal Medicine for the Treatment of Obesity: An Overview of Scientific Evidence from 2007 to 2017. *Evid. Based Complement. Alternat. Med.* **2017**, *2017*, 8943059. [CrossRef] [PubMed]
58. Mityukova, T.A.; Chudilovskaya, E.N.; Basalai, A.A. Reactivity of the Thyroid System to Short-Term Stress in Wistar Rats with Visceral Obesity and Restricted Social Activity. *J. Evol. Biochem. Physiol.* **2022**, *58*, 465–475. [CrossRef] [PubMed]
59. Sampey, B.P.; Vanhoose, A.M.; Winfield, H.M.; Freemerman, A.J.; Muehlbauer, M.J.; Fueger, P.T.; Newgard, C.B.; Makowski, L. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. *Obesity* **2011**, *19*, 1109–1117. [CrossRef] [PubMed]
60. Carvalho, K.M.; Marinho Filho, J.D.; de Melo, T.S.; Araújo, A.J.; Quetz, J.d.a.S.; da Cunha, M.d.o.P.; de Melo, K.M.; da Silva, A.A.; Tomé, A.R.; Havt, A.; et al. The Resin from *Protium heptaphyllum* Prevents High-Fat Diet-Induced Obesity in Mice: Scientific Evidence and Potential Mechanisms. *Evid. Based Complement. Altern. Med.* **2015**, *2015*, 106157. [CrossRef] [PubMed]
61. Koneru, K.; Bhatt, V.; Kakrani, A.; Edara, M.; Reddy, V.T.; Jawade, P.G. A study of non-alcoholic fatty liver disease-liver fat score in overweight and obese individuals. *J. Fam. Med. Prim. Care* **2022**, *11*, 4368–4374. [CrossRef]
62. Meherubi, I.; Nessa, A.; Huda, M.N.; Naznen, F.; Afroz, L.; Mumu, N.S.; Rukunuzzaman, M.; Yeasmin, F.; Sharmin, A.; Chowdhury, R. Relationship between Body Mass Index and Serum Alanine Transaminase Concentration in Obese Female. *Mymensingh Med. J.* **2022**, *31*, 600–605. [PubMed]
63. Albrahim, T.; Robert, A.A. Lycopene Effects on Metabolic Syndrome and Kidney Injury in Rats Fed a High-Fat Diet: An Experimental Study. *ACS Omega* **2022**, *7*, 30930–30938. [CrossRef] [PubMed]
64. Patias, S.N.; Sinhorin, V.D.G.; Sinhorin, A.P. Potential antioxidants and other biological activities of *Protium heptaphyllum* (Aubl.). March: Mini-Review. *Nat. Prod. J.* **2023**, *13*, e090223213549. [CrossRef]
65. Roman, W.A., Jr.; Piato, A.L.; Conterato, G.M.; Wildner, S.M.; Marcon, M.; Mocelin, R.; Emanuelli, M.P.; Emanuelli, T.; Nepel, A.; Barison, A.; et al. Hypolipidemic effects of Solidago chilensis hydroalcoholic extract and its major isolated constituent quercetrin in cholesterol-fed rats. *Pharm. Biol.* **2015**, *53*, 1488–1495. [CrossRef] [PubMed]
66. Santos, F.A.; Frota, J.T.; Arruda, B.R.; de Melo, T.S.; da Silva, A.A.; Brito, G.A.; Chaves, M.H.; Ra, V.S. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis.* **2012**, *11*, 98. [CrossRef] [PubMed]
67. Mannino, G.; Iovino, P.; Lauria, A.; Genova, T.; Asteggiano, A.; Notarbartolo, M.; Porcu, A.; Serio, G.; Chinigò, G.; Occhipinti, A.; et al. Bioactive triterpenes from *Protium heptaphyllum* gum resin extract exhibit cholesterol-lowering potential. *Int. J. Mol. Sci.* **2021**, *22*, 2664. [CrossRef] [PubMed]
68. Nelson, D.L.; Cox, M.M. *Princípios de Bioquímica de Lehninger*, 6th ed.; Artmed: São Paulo, Brazil, 2014.
69. Bükülmez, A.; Özer Gökaslan, Ç.; Oflu, A.T. Increase in visceral adipose tissue and subcutaneous adipose tissue thickness in children with acute pancreatitis. A case-control study. A case-control study. *Arch. Pediatr.* **2021**, *28*, 29–32. [CrossRef] [PubMed]
70. Kosmas, C.E.; Rodriguez Polanco, S.; Bousvarou, M.D.; Papakonstantinou, E.J.; Peña Genao, E.; Guzman, E.; Kostara, C.E. The Triglyceride/High-Density Lipoprotein Cholesterol (TG/HDL-C) Ratio as a Risk Marker for Metabolic Syndrome and Cardiovascular Disease. *Diagnostics* **2023**, *13*, 929. [CrossRef] [PubMed]
71. Dong, W.W.; Chen, D.; Chen, Z.; Sun, H.; Xu, Z. Antioxidant capacity differences between the major flavonoids in cherry (*Prunus pseudocerasus*) in vitro and in vivo models. *LWT* **2021**, *141*, 110938. [CrossRef]

72. Anand David, A.V.; Arulmoli, R.; Parasuraman, S. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacogn. Rev.* **2016**, *10*, 84–89. [CrossRef]
73. Xu, D.; Hu, M.J.; Wang, Y.Q.; Cui, Y.L. Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. *Molecules* **2019**, *24*, 1123. [CrossRef] [PubMed]
74. Mentxaka, A.; Gómez-Ambrosi, J.; Ramírez, B.; Rodríguez, A.; Becerril, S.; Neira, G.; Valentí, V.; Moncada, R.; Silva, C.; Unamuno, X.; et al. Netrin-1 Promotes Visceral Adipose Tissue Inflammation in Obesity and Is Associated with Insulin Resistance. *Nutrients* **2022**, *14*, 4372. [CrossRef] [PubMed]
75. Khan, I.; Lu, Y.; Li, N.; Shi, H.; Ding, L.; Hong, M.; Fang, Z. Effect of ammonia stress on AMPK regulating carbohydrate and lipid metabolism in Chinese striped-neck turtle (*Mauremys sinensis*). *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2023**, *263*, 109491. [CrossRef] [PubMed]
76. Eun Ha, C.; Bhagavan, N.V. Protein and amino acid metabolism. In *Essentials of Medical Biochemistry*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 277–322. [CrossRef]
77. Chen, J.; Li, G.; Sun, C.; Peng, F.; Yu, L.; Chen, Y.; Tan, Y.; Cao, X.; Tang, Y.; Xie, X.; et al. Chemistry, pharmacokinetics, pharmacological activities, and toxicity of Quercitrin. *Phytother. Res.* **2022**, *36*, 1545–1575. [CrossRef] [PubMed]
78. Leiherer, A.; Stoemmer, K.; Muendlein, A.; Saely, C.H.; Kinz, E.; Brandtner, E.M.; Fraunberger, P.; Drexel, H. Quercetin Impacts Expression of Metabolism- and Obesity-Associated Genes in SGBS Adipocytes. *Nutrients* **2016**, *8*, 282. [CrossRef] [PubMed]
79. Rémésey, C.; Demigné, C.; Aufrère, J. Inter-organ relationships between glucose, lactate and amino acids in rats fed on high-carbohydrate or high-protein diets. *Biochem. J.* **1978**, *170*, 321–329. [CrossRef]
80. Gu, W.; Kim, K.A.; Kim, D.H. Ginsenoside Rh1 improves high fat diet-induced obesity in mice by inhibiting adipocyte differentiation. *Biol. Pharm. Bull.* **2013**, *36*, 102–107. [CrossRef]

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CAPÍTULO 3

Effects of Extended Treatment with *Protium heptaphyllum* Liposomes on Metabolic Parameters of Obese Rats

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Effects of Extended Treatment with *Protium heptaphyllum* Liposomes on Metabolic Parameters of Obese Rats

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Simple Summary: *Protium heptaphyllum* (*P. heptaphyllum*), a plant popularly known as "almacega" or "white pitch", has been used for decades in folk medicine, mainly due to its anti-inflammatory and analgesic properties, and is already recognized due to the use of its resin. Given that obesity is considered a worldwide epidemic, associated with chronic low-grade inflammation and increased reactive oxygen species, this study investigated the effects of *P. heptaphyllum* leaves due to their richness in flavonoids and antioxidant compounds. Using a model of induced obesity in rats, the animals were treated for 28 days with liposomes containing the extract of *P. heptaphyllum* leaves. The results indicate that the treatment may have therapeutic potential not only against obesity, but also in the regulation of oxidative stress and metabolic and inflammatory parameters, possibly due to the high concentration of flavonoids present in the extract.

Abstract: *Protium heptaphyllum* (*P. heptaphyllum*), popularly known as "almacega" or "white pitch", is widely used in folk medicine due to its antioxidant, anti-inflammatory and healing properties, attributed to its richness in flavonoids and terpenes. Therefore, this study aimed to evaluate the effects of treatment for 28 days with liposomes containing *P. heptaphyllum* leaf extract in obese animals. Male Wistar rats, subjected to a hypercaloric diet for 8 weeks to induce obesity (hypercaloric chow and water enriched with 30% sucrose, ad libitum), were treated with the plant formulation (1 mg $kg^{-1}day^{-1}$, via gavage) for 28 days. The study investigated morphological, metabolic, redox state, immunological and histological parameters in adipose and liver tissue. Rats were divided into four groups: control (C), liposomes with extract (H), obese (O) and obese treated with liposomes containing extract (OH). The results indicated that the obese group (O) presented weight gain, hepatic steatosis and alterations in metabolic and inflammatory parameters. However, treatment with liposomes (OH) reduced glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine and the lipid profile. In adipose tissue, the OH group showed decreased superoxide dismutase (SOD) activity and increased glutathione S-transferase (GST) activity, in contrast to the effects observed in liver GST. In the analysis of thiobarbituric-acid-reactive substances (TBARS), it was possible to observe an increase in all groups in adipose tissue and in group O in liver tissue, in addition to a reduction in TBARS in group OH in the liver, indicating modulation of oxidative stress. The treatment also increased the concentration of IL-10 and IL-17 in the liver and decreased that of IL-6 in adipose tissue. After 28 days of treatment, these results point

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to the therapeutic potential of treatment with *P. heptaphyllum*, not necessarily only against obesity, but also an effect per se of the liposomes, possibly due to the high concentration of flavonoids present in the plant extract.

Keywords: quercetrin; phytotherapeutic treatment; oxidative stress; hypercaloric diet

1. Introduction

Protium heptaphyllum (*P. heptaphyllum*) is a plant widely found in South America, especially in the Amazon region, and is popularly known as "almacega" or "white pitch". Belonging to the Burseraceae family and the *Protium* genus, this plant stands out not only for the quality of its wood, but also for the various applications already described in traditional medicine. Recognized for its stimulating, anti-inflammatory and healing properties, it is frequently used in folk medicine. The natural substances present in *Protium heptaphyllum* increase its potential as a promising source of alternative treatments and natural derivatives, promoting benefits to human health [1,2].

The resins of the *Protium* genus are rich in flavonoids and terpenes, with emphasis on pentacyclic triterpenes. Analysis of the leaves, flowers, bark resin, stem and branches of *P. heptaphyllum* revealed that the composition found in its plant resin is rich in active substances [3]. The essential oils derived from these resins, in turn, are predominantly composed of monoterpenes [2]. The leaves are rich in flavonoids such as myricetin, quercetin and quercetin-3-β-D-glucoside [4]. The chemical diversity found in plants guides researchers to investigate the biological activity of natural products, given the capacity of these plants to serve as promising sources of active principles for the treatment of several chronic and degenerative diseases. These diseases include cardiovascular, neurological and respiratory diseases, rheumatoid arthritis, kidney diseases, and cancer, often associated with oxidative stress [5–7]. This stress is the result of increased production of free radicals and decreased antioxidant defense. Free radicals, also known as reactive oxygen species (ROS), are highly reactive molecules with one or more unpaired electrons at the center of oxygen atoms, and are produced in the cytoplasm, mitochondria or plasma membrane [8].

Another health problem that has affected millions of people is related to the dynamics of the modern world and changes in eating behavior, which stand outs as an interaction between the physical state of the organism and environmental conditions [9–11]. These changes are linked to the relationship between stress and obesity. Chronic stress is correlated with metabolic disorders and adjustments in energy homeostasis [12], and can trigger pleasurable and compulsive eating behaviors, such as a preference for sweet and fatty foods, thus contributing to the development of several diseases, such as obesity, diabetes and dyslipidemia. An increased prevalence of obesity is intrinsically associated with changes in eating patterns, sedentary lifestyles and obesogenic environments [13]. Furthermore, it is important to know that a complex interplay between genetic and external factors can act in the individual and contribute to the accumulation of adipose tissue and the development of obesity. A deeper understanding of the molecular, metabolic and endocrine mechanisms underlying obesity is essential for the development of strategies to prevent, control and/or treat this pathology. The expansion of adipose tissue contributes to the exacerbated production of adipokines and pro-inflammatory cytokines, triggering an inflammatory state that is closely linked to the development of metabolic comorbidities, such as insulin resistance, type 2 diabetes, dyslipidemia and cardiovascular diseases [14–16].

Plants and their extracts have significant importance in the treatment of various diseases, including obesity. Research suggests that the flavonoids found in these extracts can alleviate cellular stress, including neuroinflammation, oxidative stress, proteotoxicity and endoplasmic reticulum stress [17]. Furthermore, several studies demonstrate that flavonoids have beneficial effects when it comes to obesity, mainly due to their antioxidant and anti-inflammatory properties, mitigating complications related to this

pathology [18–20]. To further enhance these therapeutic effects of natural compounds, the application of nanosystems has shown to be a promising alternative. Studies have extensively investigated the use of nanosystems in the transport and optimization of the pharmacological action of natural products [21]. These systems involve different structures that vary in chemical composition, shape, charge, solubility and load capacity, among other factors [22], offering an approach that improves the solubility, stability and specific targeting of active substances, overcoming absorption and bioavailability challenges [23]. Among nanosystems, liposomes stand out, which are vesicles composed of one or more concentric phospholipid bilayers that surround an aqueous compartment. They have the capacity to transport both hydrophilic and lipophilic substances, such as drugs, biomolecules and/or diagnostic agents [24]. The incorporation of active substances into liposomes not only allows controlled release, but also minimizes side effects and enhances therapeutic effects. This represents a promising frontier for the optimization of phytotherapeutic treatments in contemporary medicine [23,25]. In addition to improving the solubility of natural compounds, this technology enhances their absorption, allowing for more effective exploration of the medicinal properties of natural ingredients. Thus, nanotechnology, especially with the use of liposomes, opens up new perspectives for the formulation of products that maximize the therapeutic potential of plant substances, contributing to significant advances in the health and well-being industry [26,27].

In view of this, and considering previous studies by our group [28] where the treatment with liposomes containing *P. heptaphyllum* leaf extract in rats under induced obesity was investigated, this study aimed to demonstrate the effects of a longer treatment, also with liposomes containing *P. heptaphyllum* leaf extract, in obese animals, in order to verify whether this route of administration for a longer period could present better effects in this experimental model.

2. Material and Methods

2.1. Extract Preparation and Liposome Development

The study was conducted at the Laboratórios Integrados de Pesquisa em Ciências Químicas (LIPEQ)—Natural Products Chemistry Laboratory and Biochemistry Laboratory, located at the Universidade Federal de Mato Grosso, Campus Universitário de Sinop. The exsiccata is cataloged under number 625 in the Herbarium Centro-Norte-Mato-Grossense (CNMT), also located at the Sinop Campus. Details on the treatment of *P. heptaphyllum* leaves and the development of the liposome containing the extract can be found in Patias et al. [28].

2.2. Experimental

2.2.1. Animals

Male Wistar albino rats were used as experimental subjects in the present study, in compliance with the conditional ethical guidelines of the Animal Use Ethics Committee of the Universidade Federal de Mato Grosso, under approval number 23108.031684/2021-21. The animals in the control group were fed a diet composed of a standard rodent chow (NUVILAB CR-1, Nuvital®, Colombo, Paraná, Brazil), while the animals in the experimental group were fed a hypercaloric diet, characterized by a high concentration of lipids, representing 24.5% of the total energy intake, in addition to having access to water containing sucrose (300 g L^{-1}). The experimental design and diet formulation followed the methodologies described by Nascimento et al. [29] and Comiran et al. [30]. The hypercaloric diet was prepared in the laboratory and included ingredients such as commercial feed (NUVILAB CR-1), casein, lard, condensed milk and cornstarch biscuits, as well as the addition of vitamins and minerals as detailed in Comiran et al. [30]. The acclimatization period lasted 2 weeks, followed by an 8-week study period. During the experimental period, the animals were kept in polypropylene boxes, with 4–5 rats/box, in an environment with temperature and light–dark cycle control (temperature of 22 ± 2 °C and a 12 h light–dark cycle). The animals had free access to water and food during all experimental protocols.

The animals were subdivided into 4 groups: Group C: Negative control. Group H: Control treated with *P. heptaphyllum* extract liposomes for 28 days. Group O: Obese. Group OH: Obese treated with *P. heptaphyllum* extract liposomes for 28 days.

In the groups subjected to treatment with liposomes of the extract (H, OH), a dose of 1.0 mg kg^{-1}, established by the Malone test [31], was administered via gavage once a day over 30 days (from the 31st to the 60th day of experiment). The control groups (C and O) were treated with saline solution (NaCl 0.9%). After the last day of the treatment and experimental protocol, the animals were fasted for 8 h to collect the blood samples and tissues. Blood collection was performed by cardiac puncture using heparinized syringes under anesthesia (Ketamine 50 mg kg^{-1} and Xilazin 2 mg kg^{-1}); then, the animals were euthanized by decapitation. Several tissues were collected by dissection, such as liver and adipose tissue, weighed to determine the absolute tissue weight (g) and relative tissue weight (g 100 g^{-1} of body weight), and frozen at −80 °C for subsequent analyses. In addition, body weight (g) was recorded to evaluate the final body weight of the animals after the treatment protocol. Plasma was obtained from the collected whole blood.

2.2.2. Characterization of Obesity, Calorie, Food and Water Consumption

The relative weight of periepididymal and retroperitoneal fat, in g/100 g of body weight, was assessed at the end of the experiment, as well as the daily consumption of water and food. The protocol to analyze the consumption of food, water and calories was the same as described in [28].

2.2.3. Intraperitoneal Insulin Tolerance Test (IPITT) and Oral Glucose Tolerance Test (OGTT)

The IPITT and OGTT were developed as described in [30]. The animals were evaluated at the end of the experimental protocol, after 28 days of treatment.

2.2.4. Metabolic Parameters in Liver, Adipose Tissue and Plasma

Aspartate aminotransferase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), amylase, lipase, creatinine, glucose, lactate, total proteins, as well as triglycerides, total cholesterol, and LDL-, HDL- and VLDL-cholesterol of blood plasma were analyzed as described in [28].

For adipose and liver tissues, glucose was determined according to Dubois et al. [32], glycogen according to Bidinotto et al. [33] and lactate was estimated according to Harrower and Brown [34]. Protein content was determined by the method described by Bradford [35], amino acid levels were determined according to Spies [36] and ammonia was measured according to the method of Gentzkow and Masen [37].

Superoxide dismutase (SOD) activity was measured according to Misra and Fridovich [38]. For catalase (CAT) activity, the decomposition of H_2O_2 was observed following Nelson and Kiesow [39]. Glutathione S-transferase (GST) activity was measured by the production of glutathione S-2,4-dinitrophenyl [40]. The dosage of the enzyme glutathione peroxidase (GPx) was performed according to the method described by Paglia and Valentine [41]. For reduced glutathione (GSH), the formation of anilide thiolate was evaluated and compared to a standard GSH curve [42], and ascorbic acid (ASA) was measured and compared to an ascorbic acid curve according to Roe [43]. The indirect markers of oxidative damage studied included thiobarbituric-acid-reactive substances (TBARS), which were quantified and compared to an increasing MDA curve [44], in addition to carbonyl proteins (Carbonyl), according to the method of Colombo et al. [45]. The protein content in the dosages, with the exception of ascorbic acid, was determined by the Bradford method [35], using bovine serum albumin as a standard for constructing the calibration curve. The absorbance readings of the samples were performed at 595 nm.

2.2.5. Histological Analyses of Liver Tissue

Liver tissue samples were fixed in buffered formalin, followed by dehydration in different concentrations of ethanol. Subsequently, the samples were embedded in resin and then hardened in Histotech plastic molds. This material was then sectioned into 3 μm cross sections (Leica Microsystems 2245 semi-automatic rotary microtome, Wetzlar, Germany) and stained with hematoxylin and eosin (H&E). The slides were then observed under a trinocular optical microscope (Motic brand, Dongguan, China) with the aid of Motic Images Plus 2.0 software, which was used to capture and analyze the images. The images of the liver were evaluated for the presence of apoptosis and/or necrosis, hepatic steatosis (micro- and macro-vesicular), tissue inflammation with the presence of leukocytes infiltration, fibrosis and hepatocyte degeneration.

2.2.6. Immunological Evaluation

To perform immunological analyses of cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 17 (IL-17) and interleukin 1β (IL-1β), the enzyme-linked immunosorbent assay (ELISA) methodology was used, using commercial kits from the DIY Elisa® brand, according to the instructions provided by the manufacturer (Labtest®, Diagnóstico S.A., Minas Gerais, Brazil).

2.3. Data Analysis

The data were initially subjected to the Kolmogorov–Smirnov normality test. Then, a two-way analysis of variance (ANOVA) was realized, and the Tukey test to assess means when more than two groups were present was conducted. When necessary, the Kruskal–Wallis test was used, and Dunn's test. Statistical significance was set at $p < 0.05$. Data were presented as mean +/− standard deviation or median and total range, depending on the analysis. Statistical analysis was performed using the Graph Pad Prism 8.0 statistical software.

3. Results

3.1. Anthropometric Measurements and Food Intake

Among the results obtained, we observed an increase in the final weight and weight gain of the animals in groups O (obese) and OH (obese treated with liposomes) compared to group C (control). The consumption of feed and water by the animals differed significantly between the control groups (C and H) and obese groups (O and OH) throughout the experimental period, with feed consumption being lower in the obese groups in relation to the control groups and water consumption being higher in the obese groups when compared to the control groups, and with significantly lower water consumption in the OH group compared to the O group, demonstrating that the liposomes of *P. heptaphyllum* reduced the consumption of water with sucrose in this group. When we analyzed the calorie calculation (kcal $day^{-1}rat^{-1}$), consumption was significantly higher in groups O and OH when compared to group C (Table 1).

As for tissue weight, we observed an increase in the weight of the periepididymal and retroperitoneal adipose tissue of animals in groups O and OH compared to group C, but obese animals treated with liposomes had a reduction in retroperitoneal tissue in relation to obese animals. The liver was shown to be enlarged in the obese group compared to the negative control, and the OH group significantly reduced the size of this organ in relation to group O, keeping it similar in size to the control (Table 1).

Through the analysis of weight evolution from the fourth week of obesity induction, it was possible to observe a significant alteration in the body weight of the rats in the obese groups, with weight being significantly higher in the obese groups (O and OH) in relation to negative group (Figure 1), but no difference was observed between the groups in relation to the treatment with liposomes (C vs. H and O vs. OH) (Figure 1).

Table 1. Food ingestion and anthropometric markers.

	Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Initial body weight (g) (1st day)	163.12 ± 9.85	178.28 ± 35.56	170.12 ± 42.22	174.12 ± 29.48
Body weight (g) (31st day)	194.31 ± 37.63	198.51 ± 34.42	200.62 ± 36.98	197.95 ± 29.89
Final body weight (g) (60th day)	349.48 ± 29.85	355.25 ± 32.09	413.58 ± 38.85 **	406.22 ± 48.57 *
Weight gain (g)	162.21 ± 13.81	148.51 ± 19.08	195.21 ± 12.66 *	201.60 ± 37.12 *
Feed consumption ($g\ day^{-1}rat^{-1}$)	23.33 ± 1.68	24.06 ± 1.59	17.55 ± 1.93 ****	18.06 ± 1.79 ****
Water intake ($mL\ day^{-1}rat^{-1}$)	36.61 ± 2.28	38.99 ± 2.43	55.31 ± 5.11 ****	52.74 ± 7.27 ****/####
Calorie intake ($kcal\ day^{-1}rat^{-1}$)	96.04 ± 15.36	94.03 ± 11.64	157.70 ± 22.29 ****	150.60 ± 36.22 ****
Periepididymal adipose tissue (g)	4.42 ± 0.69	5.07 ± 0.60	9.12 ± 1.15 ****	9.08 ± 1.58 ****
Retroperitoneal adipose tissue (g)	6.00 ± 1.22	5.10 ± 0.93	12.01 ± 2.91 ***	15.82 ± 3.39 ****/#
Liver (g)	10.77 ± 0.84	10.96 ± 1.19	13.23 ± 0.63 ****	11.58 ± 0.80 ##

Results are expressed as mean ± standard deviation. Analysis was performed by ANOVA (Two-way) and Tukey's test. * $p < 0.05$ compared with C; ** $p < 0.01$ compared with C; *** $p = 0.0001$ compared with C; **** $p < 0.0001$ compared with C; # $p < 0.05$ compared with O; ## $p < 0.01$ compared with O; #### $p < 0.0001$ compared with O.

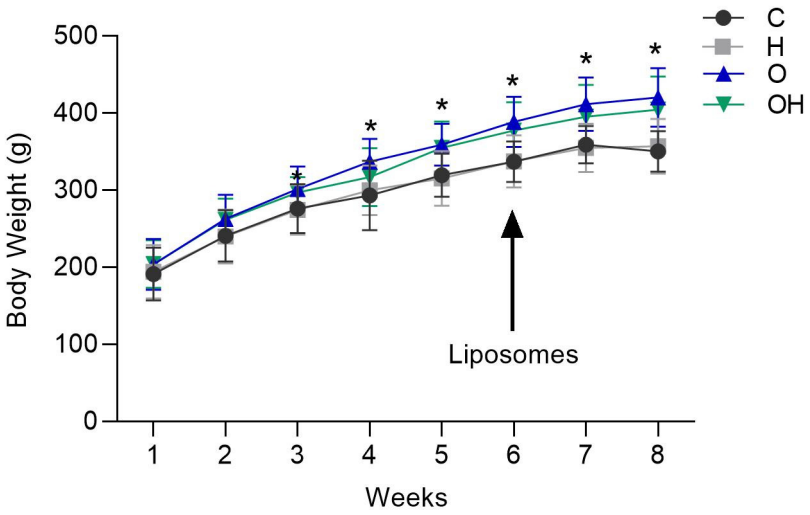

Figure 1. Weight gain of rats in the negative control (C), liposomes (H), obese (O) and obese + liposomes (OH) groups between the 1st and 8th week of the experimental design. Data are expressed as mean ± standard deviation. Analysis was performed by ANOVA (Two-way) and Tukey's test. * $p < 0.05$ compared with C; ($n = 8$).

There was no statistical difference between the groups in relation to the IPITT (Figure 2A,B), demonstrating that there was no difference in insulin sensitivity between the groups. On the other hand, when evaluating the OGTT data, a statistical difference was observed between the OH group and the C group, with results being significantly greater in the OH group (Figure 2C,D).

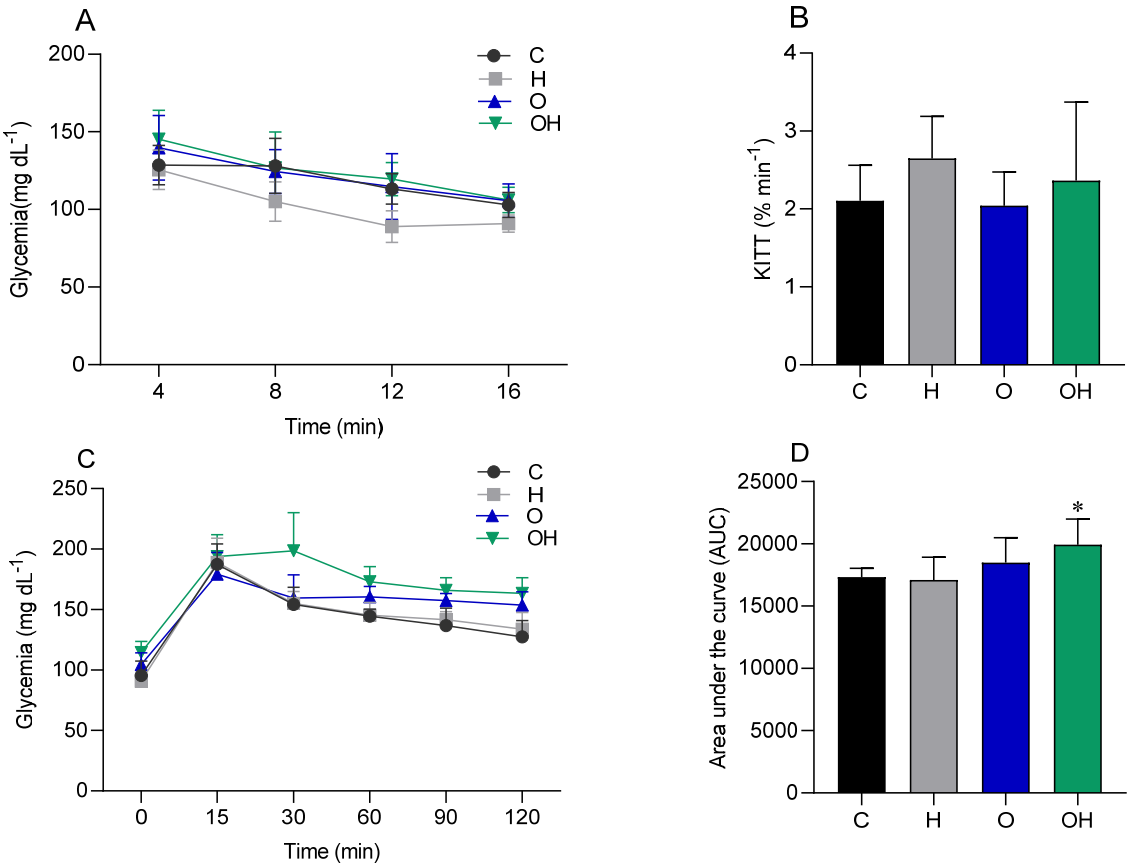

Figure 2. Glycemic curve (**A**) and glucose decay constant (KITT) (**B**), generated by the intraperitoneal insulin tolerance test (IPITT), in the control (C), liposomes (H), obese (O) and obese + liposomes (OH) groups. Glycemic curve (**C**) and area under the curve (**D**), generated by the oral glucose tolerance test (OGTT) of the control (C), liposomes (H), obese (O) and obese + liposomes (OH) groups. Data are expressed as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. * $p < 0.05$ compared with C; ($n = 8$).

3.2. Analysis of Plasma Parameters

Plasma glucose levels were elevated in groups O and OH in relation to the negative control, and the treatment with liposomes significantly decreased the glucose concentration in the treated obese group compared to the obese group. Total proteins did not change between groups. The activity of the aminotransferases (ALT, AST) and ALP enzymes reduced in the obese groups when compared to the negative control. Creatinine levels were decreased in groups H and OH compared to group C, and the OH group also had a statistically significant reduction when compared to group O. For the dosages of amylase and lipase activities, there was no statistical difference between the groups (Table 2). Regarding the lipid profile data of the animals studied, a decrease in total cholesterol and HDL cholesterol concentrations was seen in the OH group in relation to the negative control. LDL levels were decreased in groups H and OH compared to the C group. VLDL was decreased in H, O and OH compared to group C. We did not observe significant differences in dosages of triglycerides or in the calculation of the triglyceride/HDL cholesterol ratio (TG/HDL) in the groups studied (Table 2).

Table 2. Plasma biochemical parameters.

	Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Glucose (mg dL^{-1})	117.00; 53.00	121.50; 17.00	188.00; 21.00 ****	154.00 ± 55.00 ****/##
Total proteins (mg dL^{-1})	6.56; 7.68	6.55; 2.48	6.14; 3.79	63; 3.54
ALT (U L^{-1})	71.50; 40.00	60.50; 11.00	44.50; 11.00 **	40.00; 19.00 ****
AST (U L^{-1})	188.00 ± 53.37	133.90 ± 27.65	118.10 ± 43.22 *	125.30 ± 41.62 *
ALP (U L^{-1})	176.10 ± 28.29	162.50 ± 18.30	110.10 ± 14.89 ****	111.80 ± 17.89 ****
Creatinine (U L^{-1})	1.30 ± 0.19	0.86 ± 0.12 **	1.06 ± 0.31	0.65 ± 0.16 ****/##
Amylase (U L^{-1})	623.80 ± 117.30	644.50 ± 78.89	680.40 ± 61.02	713.18 ± 58.05
Lipase (U L^{-1})	25.00 ± 5.90	19.75 ± 2.65	21.63 ± 6.18	25.88 ± 3.52
Cholesterol (mg dL^{-1})	149.60 ± 17.20	130.00 ± 19.49	135.90 ± 20.67	116.10 ± 16.36 **
HDL (mg dL^{-1})	46.38 ± 6.18	40.75 ± 7.20	40.88 ± 3.27	36.38 ± 3.88 *
LDL (mg dL^{-1})	94.68 ± 20.57	73.46 ± 10.07 *	78.28 ± 11.76	73.10 ± 12.11 *
VLDL (mg dL^{-1})	21.43 ± 6.26	13.78 ± 3.18 **	13.20 ± 3.09 **	15.88 ± 2.44 **
Triglycerides (mg dL^{-1})	74.00; 93.00	72.00; 46.00	69.00; 48.00	53.00; 38.00
TG/HDL (mg dL^{-1})	1.67; 1.93	1.50; 0.67	1.75; 0.44	1.51; 1.14

Data are presented as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. For the quantification of glucose, total proteins, ALT, triglycerides and the TG/HDL ratio, the Kruskal–Wallis test was used, and Dunn's test, with data expressed as median and total range. * $p < 0.05$ compared with C; ** $p < 0.01$ compared with C; **** $p < 0.0001$; ## $p < 0.01$ compared with O; ($n = 8$).

3.3. Analysis of Redox Status Parameters in Adipose and Liver Tissue

Regarding the oxidative stress biomarkers in adipose tissue, we observed a decrease in SOD activity in the OH group in relation to the negative control and obese groups (Figure 3A). No difference was observed in catalase activity between groups (Figure 3B). The GST enzyme increased its activity in the OH group compared to the C group (Figure 3C).

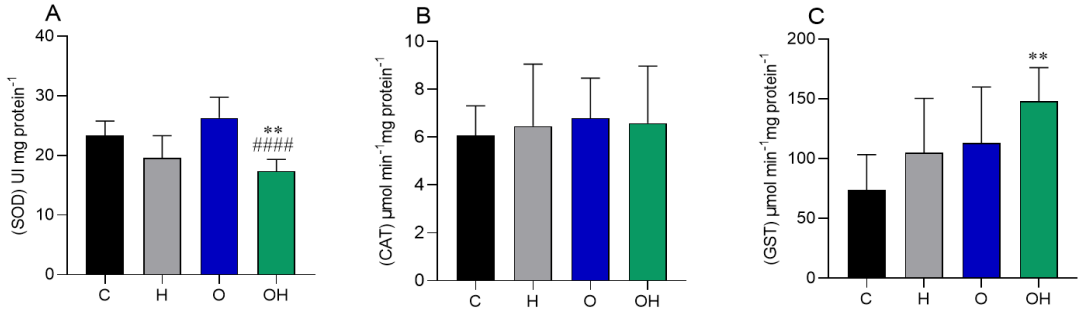

Figure 3. Influence of liposomes containing *P. heptaphyllum* extract on adipose tissue of rats subjected to a hypercaloric diet. SOD: superoxide dismutase (**A**), CAT: catalase (**B**) and GST: glutathione S-transferase (**C**). Data are expressed as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. ** $p < 0.01$ compared with C; #### $p < 0.0001$ compared with O; ($n = 8$).

In the liver tissue, there was a decrease in the activity of the GST enzyme in group H compared to group C, and in group OH compared to groups C and O (Figure 4C). The GPx enzyme decreased its activity in group H compared to group C (Figure 4D). No difference was observed in SOD and CAT activity between groups (Figure 4A,B, respectively).

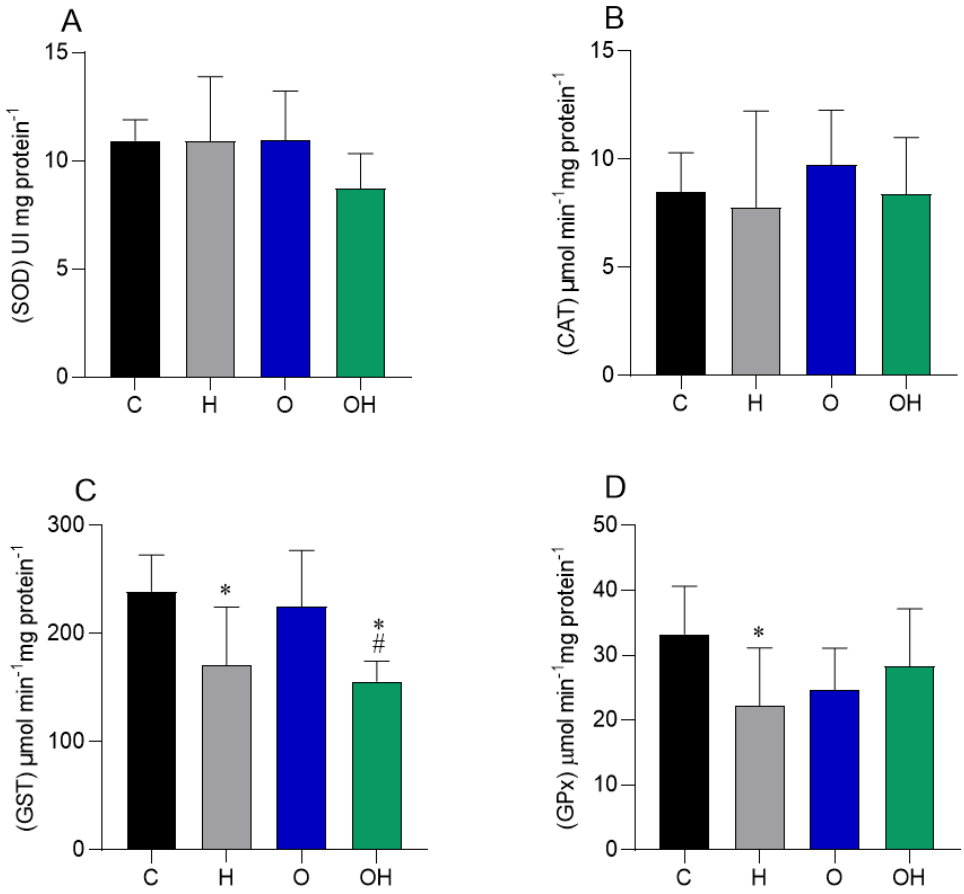

Figure 4. Influence of liposomes containing *P. heptaphyllum* extract on liver tissue of rats fed a hypercaloric diet. SOD: superoxide dismutase (**A**), CAT: catalase (**B**), GST: glutathione S-transferase (**C**) and GPx: glutathione peroxidase (**D**). Data are expressed as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. For SOD analysis, the Kruskal–Wallis test was used, and Dunn's test, with data expressed as median and total range. * $p < 0.05$ compared with C; # $p < 0.05$ compared with O; ($n = 8$).

Regarding markers of lipid damage in adipose tissue, we observed increased levels of TBARS in groups H, O and OH in relation to the negative control. There were no significant changes for the dosages of GSH, ASA and Carbonyl in adipose tissue (Table 3). On the other hand, TBARS levels increased in the liver tissue (O vs. C), but decreased in group OH compared to group O. There was also a decrease in the levels of carbonylated proteins in group O compared to group C in liver tissue. The non-enzymatic markers GSH and ASA did not show alterations in liver tissue (Table 3).

Table 3. Redox status parameters analyzed in adipose and hepatic tissues of rats subjected to a hypercaloric diet and treated for 28 days with *P. heptaphyllum* liposomes.

		Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Adipose tissue	GSH (µmol mg protein^{-1})	34.95 ± 10.42	31.42 ± 10.83	32.48 ± 11.60	21.34 ± 6.40
	ASA (µmol g tissue^{-1})	0.37 ± 0.07	0.34 ± 0.08	0.32 ± 0.05	0.32 ± 0.06
	TBARS (nmol mg protein^{-1})	0.64; 0.48	1.43; 1.56 **	1.38; 0.78 **	1.43; 1.03 **
	Carbonyl (nmol mg protein^{-1})	238.38 ± 90.30	149.77 ± 48.27	170.73 ± 78.59	157.08 ± 56.79

Table 3. *Cont.*

		Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Liver	GSH (μmol mg protein^{-1})	19.68 ± 4.20	25.85 ± 6.48	20.26 ± 5.55	15.52 ± 6.83
	ASA (μmol g tissue^{-1})	3.05 ± 0.55	2.89 ± 0.52	2.70 ± 0.27	3.24 ± 0.52
	TBARS (nmol mg protein^{-1})	0.14; 0.05	0.19; 0.13	0.24; 0.04 **	0.10; 0.12 ####
	Carbonyl (nmol mg protein^{-1})	15.65; 2.48	13.26; 6.54	12.58; 9.08 *	13.57; 4.63

Data are presented as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. For the analysis of TBARS in adipose tissue and liver tissue, as well as carbonyls, the Kruskal–Wallis test was used, and Dunn's test, with data expressed as median and total range. * $p < 0.05$ compared with C; ** $p < 0.01$ compared with C; #### $p < 0.0001$ compared with O; ($n = 8$).

3.4. Evaluation of Metabolic Parameters in Adipose and Hepatic Tissue

In the analysis of adipose tissue metabolites, we observed that glucose concentration reduced in the obese rats treated with liposomes compared to the negative control. Glycogen decreased in groups H, O and OH, also compared to group C. For ammonia dosage, we verified an increase in the obese rats and also in the rats treated only with liposomes when compared to the C group, but the treatment reduced this parameter in group OH in relation to group O. Regarding the levels of amino acids, lactate and total proteins, no changes were observed between groups in adipose tissue (Table 4). In addition, we verified an increase in glucose concentration in liver tissue in all groups compared to group C. The concentration of amino acids decreased in groups O and OH in relation to group C. The concentration of ammonia decreased in all treatments in relation to the negative control. Lactate decreased in group O when compared to the C group. For total proteins and glycogen dosages, there were no changes between treatments (Table 4).

Table 4. Analysis of metabolites in rat tissues (liver and adipose tissue) subjected to obesity and treated with liposomes.

		Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Adipose Tissue	Glucose (μmol g tissue^{-1})	5.28 ± 1.71	2.93 ± 1.87	3.73 ± 2.25	2.69 ± 1.57 *
	Glycogen (μmol g tissue^{-1})	11.44 ± 2.16	6.59 ± 1.85 ***	6.15 ± 1.40 ***	5.52 ± 2.93 ****
	Lactate (μmol g tissue^{-1})	1.59; 1.32	1.25; 1.77	1.74; 1.25	2.24; 0.90
	Total Proteins (mg mL^{-1})	0.55 ± 0.33	0.64 ± 0.32	0.62 ± 0.35	0.61 ± 0.37
	Aminoacids (mmol g tissue^{-1})	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
	Ammonia (μmol g tissue^{-1})	0.38; 0.55	1.28; 0.25 ***	1.22; 0.33 **	0.50; 0.46 #
Liver	Glucose (μmol g tissue^{-1})	33.93 ± 10.17	69.96 ± 15.80 **	87.22 ± 22.29 ****	69.59 ± 20.00 **
	Glycogen (μmol g tissue^{-1})	1.58; 0.54	1.89; 1.37	1.68; 0.83	2.06; 1,40
	Lactate (μmol g tissue^{-1})	1.62; 0.87	1.73; 1.66	0.89; 0.85 *	1.29; 0.37
	Total Proteins (mg mL^{-1})	6.67; 0.72	7.08; 7.28	6.84; 4.77	8.14; 8.57
	Aminoacids (mmol g tissue^{-1})	0.108 ± 0.014	0.103 ± 0.015	0.074 ± 0.008 ***	0.061 ± 0.010 ****
	Ammonia (μmol g tissue^{-1})	1.16 ± 0.24	0.74 ± 0.16 **	0.86 ± 0.21 *	0.58 ± 0.24 ****

Data are presented as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. For the evaluation of ammonia and lactate in adipose tissue, as well as glycogen, lactate and proteins in liver tissue, the Kruskal–Wallis test was applied, and Dunn's test, with data expressed as median and total range. * $p < 0.05$ compared with C; ** $p < 0.01$ compared with C; *** $p < 0.001$ compared with C; **** $p < 0.0001$ compared with C; # $p < 0.05$ compared with O; ($n = 8$).

3.5. Analysis of Cytokines in Adipose and Hepatic Tissues

No changes were observed in the levels of TNF-α and IFN-γ in adipose and liver tissues, as well as no changes in the levels of IL-10, IL-17 and IL-1β in adipose tissue. However, in adipose tissue, IL-6 levels were reduced in groups H, O and OH compared to

group C. In liver tissue, an increase in IL-10 was observed in groups H and OH compared to group C, and an increase in IL-17 in groups O and OH compared to group C. No changes were detected in the levels of TNF-α, IFN-γ, IL-6 and IL-1β in liver tissue (Table 5).

Table 5. Evaluation of cytokines TNF-α, IFN-γ, IL-6, IL-10, IL-17 and IL-1β in adipose and liver tissues.

		Negative Control (C)	Liposomes (H)	Obese (O)	Obese + Liposomes (OH)
Adipose Tissue	TNF-α (pg mL^{-1})	3.20 ± 0.33	3.24 ± 0.37	3.10 ± 0.35	3.27 ± 0.25
	IFN-γ (pg mL^{-1})	3.43 ± 0.20	3.39 ± 0.25	3.09 ± 0.35	3.13 ± 0.30
	IL-6 (pg mL^{-1})	3.69 ± 0.13	3.26 ± 0.25 *	3.14 ± 0.29 **	3.22 ± 0.22 **
	IL-10 (pg mL^{-1})	2.65 ± 0.27	2.58 ± 0.27	2.55 ± 0.23	2.68 ± 0.36
	IL-17 (pg mL^{-1})	3.65 ± 0.17	3.58 ± 0.23	3.62 ± 0.16	3.68 ± 0.12
	IL-1β (pg mL^{-1})	2.59 ± 0.25	2.62 ± 0.42	2.61 ± 0.27	2.79 ± 0.23
Liver	TNF-α (pg mL^{-1})	4.50 ± 0.05	4.47 ± 0.05	4.57 ± 0.07	4.51 ± 0.04
	INF-γ (pg mL^{-1})	4.32 ± 0.09	4.37 ± 0.11	4.43 ± 0.09	4.46 ± 0.12
	IL-6 (pg mL^{-1})	4.32 ± 0.03	4.36 ± 0.05	4.37 ± 0.06	4.40 ± 0.10
	IL-10 (pg mL^{-1})	2.87 ± 0.31	3.17 ± 0.05 **	3.13 ± 0.03	3.20 ± 0.03 ****
	IL-17 (pg mL^{-1})	5.62 ± 0.04	5.62 ± 0.02	5.66 ± 0.03 *	5.66 ± 0.02 *
	IL-1β (pg mL^{-1})	2.15 ± 0.32	2.44 ± 0.26	2.49 ± 0.38	2.44 ± 0.18

Data are shown as mean ± standard deviation. Analysis was performed by ANOVA (two-way) and Tukey's test. For the evaluation of IL-6 in adipose tissue and IL-10 and IL-1β in liver tissue, the Kruskal–Wallis test was applied, and Dunn's test, with data expressed as median and total range. * $p < 0.05$ compared with C; ** $p < 0.01$ compared with C; **** $p < 0.0001$ compared with C. For statistical analysis, data were logarithmically transformed; ($n = 8$).

3.6. Histopathological Evaluation in Liver

Animals fed standard diets (C and H) exhibited a liver with well-formed nucleated hepatocytes, an adequate sinusoidal matrix and an absence of signs such as inflammatory infiltrate, fibrosis or lipid retention, suggesting an architecture compatible with normal liver function (Figure 5, C and H). For all obese rats, it was possible to observe mild steatosis (Figure 5, O and OH).

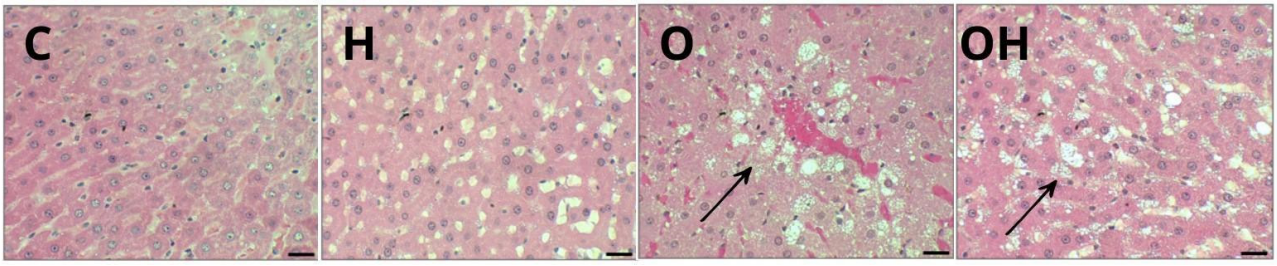

Figure 5. Photomicrograph of a sample of one specimen from each group of liver tissue, stained with hematoxylin and eosin (H&E), showing several morphological alterations in groups C (control), H (Liposomes), O (obese) and OH (Obese + Liposomes). Steatosis: lipid vacuoles in the adipose cytoplasm (arrows) in groups O and OH. Resolution: Bar 30 μm.

4. Discussion

The therapeutic potential of *P. heptaphyllum* liposomes, rich in flavonoids, was evaluated in this study, which revealed important effects on induced obesity in animals, such as a reduction in liver tissue weight, reductions in glucose, cholesterol, LDL-cholesterol and VLDL-cholesterol in the serum, and a reduction in TBARS levels in the liver. Furthermore, our data presented an increase in final body weight and weight gain of animals in the obese groups when compared to the control group, confirming the success of obesity induction in

this model induced by a diet rich in lipids and carbohydrates. Food consumption was reduced in the obese groups throughout the experimental period. However, the consumption of sucrose-rich water was higher in the obese animals, group O, and the administration of liposomes was effective in reducing this consumption in the animals of the OH group. Despite the lower food consumption, the calorie calculation ($kcal day^{-1} rat^{-1}$) was higher in the groups that received hypercaloric food, which is consistent with findings from other researchers [28,46–49].

The results demonstrated that obesity significantly influences the weight of animals, food consumption and distribution of adipose tissue, with an increase in the weight of periepididymal and retroperitoneal adipose tissue in rats subjected to obesity. This corroborates the idea that obesity affects the distribution of adipose tissue, promoting the accumulation of white adipose tissue, mainly visceral, such as retroperitoneal tissue, which is very important in the development of diabetes. Silva et al. [50] and Ribeiro et al. [51], using the same obesity induction model, also observed an increase in body weight and accumulation of visceral white adipose tissue in male Wistar rats. In the present study, prolonged treatment with liposomes did not significantly alter body weight, weight gain or periepididymal adipose tissue compared to the respective control groups (C and O). However, the treatment increased the amount of retroperitoneal adipose tissue in the OH group compared to the obese group. Similar results were observed in studies by Carvalho et al. [52,53], who investigated the effects of α, β-amyrin and *P. heptaphyllum* resin in obese mice induced by a high-fat diet. Both studies, which administered doses of 10 and 20 mg kg^{-1} for 15 weeks, demonstrated a lower increase in body weight, reduced visceral fat accumulation and a significant reduction in net energy intake, suggesting that prolonged treatment with *P. heptaphyllum* liposomes may have beneficial effects in controlling obesity and its metabolic complications, mainly due to the antioxidant and anti-inflammatory properties of the flavonoids present [18,19].

The obese group showed a significant increase in liver weight compared to the control group, along with hepatomegaly, hepatic steatosis (confirmed by histological analysis), increased hepatic glucose and oxidative stress with elevated lipid peroxidation. Treatment with liposomes was effective in significantly reducing liver size compared to the untreated obese group and in decreasing lipid peroxidation, although it did not alter hepatic glucose levels or the steatosis observed histologically. In a previous study using *P. heptaphyllum* liposomes for 14 days [28], such a reduction in liver size and hepatic TBARS levels was not observed, but in the 28-day study, the treatment was more effective, suggesting a time-dependent effect of liposomes in the treatment of obesity and its comorbidities.

No significant differences were observed among groups in relation to the IPITT, which suggests that, even with the increase in body weight in the obese groups, the insulin response may not have been affected at that specific time. When evaluating the OGTT results, the results did not differ between the C vs. H and C vs. O groups, demonstrating that the pancreas of these animals maintains good insulin secretory activity. Furthermore, the absence of statistically significant differences between these groups (C, H and O) during the OGTT indicates that, at that point, no marked differences were observed between them in the body's ability to metabolize glucose. However, in the OGTT test, it was also observed that treatment with liposomes in the obese group (OH) generated glucose intolerance when compared to the control group. It is possible that the association of obesity with treatment with liposomes generated a synergistic effect leading to glucose intolerance and greater difficulty for the animals in this OH group to secrete insulin when faced with a glucose overload.

Although these results were observed in the IPITT and OGTT, it is important to emphasize that in the fasting state, obese animals presented significant hyperglycemia and that treatment with *P. heptaphyllum* liposomes was effective in correcting this glycemic alteration in OH animals. This suggests a potentially beneficial effect of the extract in reducing plasma glucose in obese animals, and these data are consistent with previous studies with *P. heptaphyllum*, which may be related to a higher level of circulating insulin [4,52–54].

Otherwise, in the study by Patias et al. [28] where the treatment of obese animals was for 14 days, glucose levels did not reduce, which leads us to suggest that a longer treatment time is necessary for this hypoglycemic effect to appear (28 days or more). However, as in that study, amylase and lipase, important digestive enzymes, were not modified.

Another parameter that is frequently altered in an obesity condition is the lipidic profile, once the obese frequently presents dyslipidemia. However, we did not observe significant differences between C and O groups in the present work. On the other hand, it was demonstrated that the treatment with liposomes of *P. heptaphyllum* was effective in presenting a hypocholesterolemic effect, reducing significantly the levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol. The treatment is really significant once it can protect the animals from the development of atherosclerosis. Regarding the triglyceride levels, no difference was observed between groups. These beneficial effects may be related to the presence of flavonoids in *P. heptaphyllum*, such as quercetin-3-β-D-glucoside, myricetin and quercetrin, which are known for their antioxidant properties and other biological effects. Previous studies highlight the role of these bioactive compounds [55,56]. In particular, quercetrin promotes important cardioprotective effects in experimental models exposed to a high-calorie diet [57].

Although triglycerides and the triglycerides/HDL ratio did not change in this experimental model, these findings are interesting because they show that this treatment time resulted in improvements in the lipid profile of the rats in general. When we look at the findings of the 14-day study [28], the results showed some similarities, such as a reduction in LDL and VLDL for groups treated with liposomes. On the other hand, there were no changes in total cholesterol and HDL, but the triglyceride/HDL ratio had an elevation in the obese group treated with liposomes, showing that 14 days was not enough to alleviate these changes. In a study by Santos et al. [54], they emphasized the hypoglycemic effects on the lipid profile obtained by using a mixture of α, β-amyrin extracted from *P. heptaphyllum,* which seemed more prominent at 100 mg/kg, with significant reductions in VLDL and LDL cholesterol and an elevation in HDL cholesterol.

When assessing markers of liver, hepatobiliary and kidney damage, we can observe that all enzymes had their activities reduced in the groups with induced obesity. For creatinine, the groups containing liposomes had a reduction in this marker and it was more reduced in the treated obese group than in the untreated obese group. These findings are in line with the work of Patias et al. [28], as they also observed this reduction in addition to those in aminotransferases and ALP in the obese groups. However, the group treated only with liposomes for AST activity showed an effect per se that did not appear at 28 days. In the current study, on the other hand, at 28 days of treatment, a reduction in creatinine appeared in the treated obese individuals when compared to the untreated obese individuals, which leads us to suggest that with the increase in treatment time, the chance of kidney damage decreases. The increase in aminotransferases and ALP levels is generally associated with liver damage or inflammation, and their decrease may be associated with problems in the production of these enzymes or specific nutritional deficiencies [58,59]. However, as there was induction with a diet poorer in proteins, the decrease is also justified by the lower presence of essential amino acids, necessary for endogenous protein synthesis [60]. Studies carried out by Oliveira et al. [61] showed that *P. heptaphyllum* resin has a hepatoprotective effect in rats intoxicated with acetaminophen, counteracting the increases in ALT and AST, with similar efficacy to N-acetylcysteine (NAC), and also, the investigation done by Patias et al. [4] showed the effects of leaf extract from *P. heptaphyllum* (ethyl acetate fraction) in promoting a reduction in ALT and AST activities in a model of acetaminophen-induced liver injury. Creatinine is the product of muscle phosphocreatine metabolism, and its concentration is related to the use of this substrate by the muscle during vigorous muscle contraction [60], and decreased creatinine levels are also related to other factors that can affect renal and muscle function, such as specific medical conditions, dehydration, medication intake and other individual variations [62].

Adipose tissue is essential in regulating the pathophysiological mechanisms of obesity [63,64]. However, in this study, we did not observe intense changes in enzymatic activities compared to the experimental model examined, but it was possible to observe a reduction in the treated obese group (OH) in SOD and an increase in this same group in GST. In line with this view, the flavonoids present in *P. heptaphyllum* [55], known for their antioxidant and anti-inflammatory properties, can reduce the production of ROS and interfere with enzymatic antioxidant activities [52,53]. The enzymatic changes observed here suggest antioxidant modulation; however, there was an increase in the lipid damage marker (TBARS), suggesting increased oxidative stress in the adipose tissue of these OH animals, and the active ingredients present in the liposomes were not sufficient to neutralize this effect, as observed in 14-day studies with liposomes from the plant [28]. This effect can compromise cellular structures and antioxidant mechanisms, aggravating complications associated with obesity [65]. Studies that used dietary supplementation with antioxidants, such as hydroxy-selenomethionine, showed similar results, where the increase in TBARS was counteracted after the stressor, indicating that different patterns of response to oxidative stress can be modulated depending on the concentration and type of antioxidant present [66].

Mitochondrial dysfunction and chronic inflammation in adipose tissue, exacerbated by oxidative stress, are crucial factors in worsening metabolic complications [67–69]. Although no protein damage or changes in non-enzymatic antioxidant parameters (GSH and ASA) were observed, treatment with liposomes for 28 days appears to have prevented the elevation of TBARS levels, unlike what was seen in a previous study with an obese group treated for 14 days [28]. This may be explained by the increase in GST in the OH group, something that was not observed in the 14-day study mentioned above [28], as flavonoids reduce oxidative stress, a key factor in obesity, by increasing antioxidant defenses and reducing inflammation [67].

Obesity may interfere with glucose uptake via the GLUT4 transporter in adipose tissue. In the present study, we observed a reduction in glycogen in the obese groups and also in the H group, in addition to a reduction in glucose levels in the OH group. Although total proteins and amino acids did not show significant variations in the groups studied, we noted a considerable increase in ammonia concentration in the obese and liposome groups, while in the OH group, this concentration was reduced compared to the O group. Considering the possible limited use of glucose as an energy source for the adipocyte, it is possible that part of the energy for this cell comes from the action of oxidative deamination by glutamate dehydrogenase. This process uses glutamate as a substrate and generates α-ketoglutarate, NADH+H^+ and ammonium ions, the last of which may be releasing its proton into the medium, thus producing ammonia [60]. We do not have findings in the scientific literature that show the effect of flavonoids on ammonia metabolism in adipose tissue, but the study by Patias et al. [28] showed a similar effect, where animals treated with liposomes per se and obese animals showed an increase in ammonia concentration. Notably, when obese animals were treated with liposomes for 14 days, levels of this biomarker were reduced. This result suggests that *P. heptaphyllum* liposomes, possibly due to their ability to improve the bioavailability of active compounds, can influence ammonia metabolism, promoting its reduction in obese states. However, the exact mechanism of interaction with flavonoids still needs to be clarified, although it is already known that they affect the liver, adipose tissue and nervous system, influencing metabolic and signaling pathways related to obesity [70].

Regarding immunological markers, the decrease in IL-6 in treated obese groups indicates a possible downregulation of inflammation, which is in line with previous findings that emphasize that *P. heptaphyllum* resins and leaves exhibit anti-inflammatory activity [28], such as in studies by Siane et al. [71]. Other research has shown that *P. heptaphyllum* extracts can reduce plasmatic concentrations of pro-inflammatory cytokines (TNF-α and IL-6), which are generated by oxidative stress and metabolic dysfunction in obesity, but this effect was not found in this study [52]. Although we did not observe reduced levels of

TNF-α, this is consistent with other research that has shown that certain flavonoids, such as those present in blueberry extract, may exert selective anti-inflammatory effects, acting on adipose tissue and specific mediators, such as IL-6, rather than TNF-α. This selectivity may be related to factors such as the dosage or bioavailability of the compounds [72].

The liver is a key organ in the process of metabolizing various exogenous and endogenous substances, and in an obese condition, it becomes vulnerable to changes. When we observed our results, we noticed that GPx and GST had similar reductions in their activities in the H group compared to the control group, and for GST, there was a reduction in the obese group treated with liposomes, which differed from the control and the obese group. Furthermore, the obesity model induced in rats caused an increase in TBARS, indicating increased lipid peroxidation. However, these levels were reduced in the OH group, but with no effect on ASA, SOD and CAT. GST is a very important enzyme in the detoxification of xenobiotics, and like GPx, it is responsible for the detoxification of lipid hydroperoxides (LOOH) [73]. The increase in TBARS seen in obese individuals, reflecting increased oxidative stress and lipid damage, found at reduced levels in the obese group, suggests that treatment with *P. heptaphyllum* liposomes may be able to protect against oxidative stress generated by obesity, as shown in studies linking obesity to an increase in oxidative biomarkers (MDA and LOOH) [74]. GST and GPx activity were also reduced in the H group per se, indicating a possible deficiency in the detoxification of substances and neutralization of hydrogen peroxide as emphasized by Zang et al. [75]. Unlike the study by Patias et al. [28], which found no changes in the activities of hepatic enzymatic antioxidants (SOD, GPx, CAT and GST), non-enzymatic antioxidants (GSH and ASA) and no changes in protein damage (protein carbonylation assay), our 28-day study with liposomes revealed changes in enzymes (GST and GPx), GSH levels and protein carbonylation. This suggests that a longer period of treatment may be necessary to impact liver redox status parameters.

Obesity can also generate increases in glucose in the liver tissue, as a possible route, due to the presence of glycerol from adipose tissue, which can be obtained from the triacylglycerol cycle that produces glucose through hepatic gluconeogenesis [60] but without being transformed into glycogen, as can be observed in the obese groups. In addition, amino acid levels were reduced in the obese groups. In this context, we noticed a decrease in ammonia concentration in the obese and liposome-treated groups, which may reflect a lower activation of the urea cycle. The effects observed per se in group H, namely the increase in glucose dosage as well as the reduction seen in ammonia, suggest that the flavonoids present, mainly quercetin-3-β-glucoside, which contains a sugar residue, may be involved in this increase [55]. As for ammonia, the observed reduction indicates an improvement in the liver's ability to eliminate toxic compounds, just as the decrease in lactate for the treated obese groups suggests a complex metabolic modulation leading to metabolic stress or adaptation [76]. These findings are consistent with those observed by Patias et al., who also reported similar results in animals treated for 14 days [28]. However, liposomes showed a positive effect in restoring lactate levels in obese individuals, which reinforces the importance of liposome treatment in modulating metabolic parameters altered by obesity.

Considering the already described antioxidant and anti-inflammatory properties of flavonoids present in *P. heptaphyllum*, and their ability to modulate enzymatic antioxidant activities, controlling ROS production, we suggest that the action of the compounds present in the liposomes in this study can reduce oxidative stress and the formation of toxic metabolites, possibly through the suppression of liver cytochrome P450 [52,53,55,77]. Previous studies with *P. heptaphyllum* have demonstrated its therapeutic action in the treatment of liver diseases [4,78,79], corroborating the idea that its bioactive compounds exert hepatoprotective effects. We observed in the liver tissue an increase in IL-17 only in the treated and untreated obese groups, suggesting a chronic inflammatory process activated in this tissue, in this condition. However, we also observed an increase in IL-10, a potent anti-inflammatory cytokine, in the liver of the groups treated with liposomes, which indicates an anti-inflammatory action of this extract even in the absence of obesity. The increase in IL-10 indicates a modulation of the immune system, potentially protecting against chronic

liver inflammation while trying to control liver stress [80,81]. These findings highlight the potential of *P. heptaphyllum* in the prevention and treatment of obesity-related and non-obesity liver diseases. Furthermore, the administration of the flavonoids discussed has shown to be a promising strategy to increase the bioavailability of and more efficiently regulate pro-inflammatory cytokines, such as IL-17, offering even greater therapeutic potential in inflammatory diseases [82].

A distinctive feature of this study was the administration of a relatively low dose of 1 mg/kg of the extract, in liposomal form, for a period of 28 days. Even with this reduced dosage, the results were significant, especially when compared to previous studies that used higher doses of flavonoids or amyrins, such as those by Carvalho [52,53] and Patias [4], whose doses ranged from 10 to 20 mg/kg and 100 mg/kg, respectively. The observed efficacy reinforces the potential of *P. heptaphyllum* liposomes, suggesting that this formulation enhances therapeutic effects, offering a promising and innovative approach in the treatment of obesity.

5. Final Considerations

Our study revealed that obesity induces significant changes in hepatic metabolism and inflammatory response, as evidenced by increased glucose and ammonia levels, and by changes in the activity of antioxidant enzymes such as GPx and GST. Flavonoids present in *P. heptaphyllum* liposomes appear to positively influence oxidative processes in adipose and hepatic tissues, with decreased SOD activity and increased GST, in addition to variations in lipid damage markers. The decrease in IL-17 and increase in IL-10 in the groups treated with *P. heptaphyllum* suggest a positive modulation of the inflammatory response, potentially protecting the liver from chronic inflammation and helping to control hepatic stress. These findings suggest the need for further investigations to explore in more depth the therapeutic role of this treatment in order to control and treat complications caused by obesity.

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References

1. Islam, R.; Kabir, M.F.; Alam, R.; Dahr, R.; Rana, M.N.; Islam, E.; Parvin, S.; Hossain, A. Sedative, membrane stability, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall. *Asian Pac. J. Trop. Dis.* **2014**, *4*, 928–933. [CrossRef]
2. Marques, D.D.; Sartori, R.A.; Lemos, T.L.G.; Machado, L.L.; Souza, J.S.N.D.; Monte, F.J.Q. Composição química dos óleos essenciais de duas subespécies de *Protium heptaphyllum*. *Acta Amaz.* **2010**, *40*, 227–230. [CrossRef]

3. Tafurt-García, G.; Muñoz-Acevedo, A. Metabolitos volátiles presentes en *Protium heptaphyllum* (Aubl.) March. colectado en Tame (Arauca-Colombia). *Boletín Latinoam. Caribe Plantas Med. Aromát.* **2012**, 11, *223–232*. Available online: https://pesquisa.bvsalud.org/portal/resource/pt/lil-647661 (accessed on 24 June 2024).
4. Patias, N.S.; Sinhorin, V.D.G.; Moura, F.R.; Cunha, A.P.F.; Lima, R.R.S.; Costa, R.J.; Costa, T.B.; Cavalheiro, L.; Bicudo, R.C.; Sinhorin, A.P. Identification of flavonoids by LC-MS/MS in leaves extract from *Protium heptaphyllum* (Aubl.) March and antioxidant activity in mice. *Nat. Prod. J.* **2021**, *11*, 715–727. [CrossRef]
5. Anmol, A.; Aggarwal, G.; Sharma, M.; Singh, S.; Sharma, U. Journey of plants natural products from plants to bioactive products. *ChemRxiv* **2022**. *preprint*. [CrossRef]
6. Chopra, B.; Dhingra, A.K. Natural products: A lead for drug discovery and development. *Phytother. Res. PTR* **2021**, *35*, 4660–4702. [CrossRef]
7. Shen, Y.; Hao, X. Natural product sciences: An integrative approach to the innovations of plant natural products. *Sci. China Life Sci.* **2020**, *63*, 1634–1650. [CrossRef]
8. Voronkova, Y.S.; Voronkova, O.S.; Gorban, V.A.; Holoborodko, K.K. Oxidative stress, reactive oxygen species, antioxidants: A review. *Ecol. Noospherology* **2018**, *29*, 52–55. [CrossRef]
9. Cai, Y.; Liu, P.; Xu, Y.; Xia, Y.; Peng, X.; Zhao, H.; Chen, Q. Biomarkers of obesity-mediated insulin resistance: Focus on microRNAs. *Diabetol. Metab. Syndr.* **2023**, *15*, 167. [CrossRef]
10. Hanson, P.; Weickert, M.O.; Barber, T.M. Obesity: Novel and unusual predisposing factors. *Ther. Adv. Endocrinol. Metab.* **2020**, *11*, 2042018820922018. [CrossRef]
11. Razzoli, M.; Pearson, C.; Crow, S.; Bartolomucci, A. Stress, overeating, and obesity: Insights from human studies and preclinical models. *Neurosci. Biobehav. Rev.* **2017**, *76*, 154–162. [CrossRef] [PubMed]
12. Morera, L.P.; Marchiori, G.N.; Medrano, L.A.; Defagó, M.D. Stress, Dietary Patterns and Cardiovascular Disease: A Mini-Review. *Front. Neurosci.* **2019**, *13*, 1226. [CrossRef]
13. Spencer, S.J.; Korosi, A.; Layé, S.; Shukitt-Hale, B.; Barrientos, R.M. Food for thought: How nutrition impacts cognition and emotion. *npj Sci. Food* **2017**, *1*, 7. [CrossRef] [PubMed]
14. Goodarzi, M.O. Genetics of obesity: What genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol.* **2018**, *6*, 223–236. [CrossRef] [PubMed]
15. Marques, D.O.; Quintilio, M.S.V. Pharmacology of obesity and risks of weight loss drugs. *Rev. Col. Cient.* **2021**, *5*, 38–49. [CrossRef]
16. Unamuno, X.; Gómez-Ambrosi, J.; Rodríguez, A.; Becerril, S.; Frühbeck, G.; Catalán, V. Adipokine dysregulation and adipose tissue inflammation in human obesity. *Eur. J. Clin. Investig.* **2018**, *48*, e12997. [CrossRef]
17. Devi, S.; Kumar, V.; Singh, S.K.; Dubey, A.K.; Kim, J.J. Flavonoids: Potential Candidates for the Treatment of Neurodegenerative Disorders. *Biomedicines* **2021**, *9*, 99. [CrossRef]
18. Nani, A.; Murtaza, B.; Sayed Khan, A.; Khan, N.A.; Hichami, A. Potencial antioxidante e anti-inflamatório dos polifenóis contidos na dieta mediterrânea na obesidade: Mecanismos moleculares. *Molecules* **2021**, *26*, 985. [CrossRef]
19. Martín, M.Á.; Ramos, S. Flavonoides dietéticos e sinalização de insulina em diabetes e obesidade. *Cells* **2021**, *10*, 1474. [CrossRef]
20. Shabrova, E.V.; Tarnopolsky, O.; Singh, A.P.; Plutzky, J.; Vorsa, N.; Quadro, L. Insights into the Molecular Mechanisms of the Anti-Atherogenic Actions of Flavonoids in Normal and Obese Mice. *PLoS ONE* **2011**, *6*, e24634. [CrossRef]
21. Pires, J.; Cargnin, S.T.; Costa, S.A.; Sinhorin, V.D.G.; Damazo, A.S.; Sinhorin, A.P.; Bicudo, R.C.; Cavalheiro, L.; Valladão, D.M.S.; Pohlmann, A.R.; et al. Healing of dermal wounds property of *Caryocar brasiliense* oil loaded polymeric lipid-core nanocapsules: Formulation and in vivo evaluation. *Eur. J. Pharm. Sci.* **2020**, *150*, 105356. [CrossRef] [PubMed]
22. Chaves, J.B.; de Moraes, B.P.T.; Ferrarini, S.R.; Fonseca, F.N.; Silva, A.R.; Albuquerque, C.F.G. Potential of nanoformulations in malaria treatment. *Front. Pharmacol.* **2022**, *13*, 999300. [CrossRef] [PubMed]
23. Tong, Y.; Xu, S.; Huang, L.; Chen, C. Obesity and insulin resistance: Pathophysiology and treatment. *Drug Discov. Today* **2022**, *27*, 822–830. [CrossRef]
24. Zanco, P.; Ferrarini, S.R.; Cruz, L.; Ferreira, L.M.; Bicudo, R.D.C.; Cavalheiro, L.; Vieira Júnior, G.M.; Sugui, M.M. Improved antimutagenic effect of *Pyrostegia venusta* (Ker Gawl.) Miers nanostructured extract in liposome and polymeric nanoparticle. *Braz. J. Pharm. Sci.* **2022**, *58*, e20234. [CrossRef]
25. Chaves, M.A.; Ferreira, L.S.; Baldino, L.; Pinho, S.C.; Reverchon, E. Current Applications of Liposomes for the Delivery of Vitamins: A Systematic Review. *Nanomaterials* **2023**, *13*, 1557. [CrossRef]
26. Quadros, C.C.; Carvalho, A.S.A.; Machado, J.L.; Michelon, M.; Salas-Mellado, M.d.L.M. Lipid Nanocarriers as an Alternative for the Delivery of Bioactive Compounds Beneficial to Health. *Curr. Bioact. Compd.* **2023**, *19*, e060323214403. [CrossRef]
27. Dutt, Y.; Pandey, R.P.; Dutt, M.; Gupta, A.; Vibhuti, A.; Raj, V.S.; Chang, C.; Priyadarshini, A. Liposomes and phytosomes: Nanocarrier systems and their applications for the delivery of phytoconstituents. *Coord. Chem. Rev.* **2023**, *491*, 0010–8545. [CrossRef]
28. Patias, N.S.; Queiroz, E.A.I.F.; Ferrarini, S.R.; Bomfim, G.F.; Aguiar, D.H.; Sinhorin, A.P.; Bello, A.A.; Silva, G.V.F.; Cavalheiro, L.; Sinhorin, V.D.G. Effect of liposomal extract of *Protium heptaphyllum* (Alb.) March in the treatment of obesity induced by a high-calorie diet. *Biology* **2024**, *13*, 535. [CrossRef]
29. Nascimento, A.F.; Sugizaki, M.M.; Leopoldo, A.S.; Lima-Leopoldo, A.P.; Luvizotto, R.A.; Nogueira, C.R.; Cicogna, A.C. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arq. Bras. Endocrinol. Metabol.* **2008**, *52*, 968–974. [CrossRef]

30. Comiran, P.K.; Ribeiro, M.C.; Silva, J.H.G.; Martins, K.O.; Santos, I.A.; Chiaradia, A.E.F.; Silva, A.Z.; Dekker, R.F.H.; Barbosa-Dekker, A.M.; Alegranci, P.; et al. Botryosphaeran Attenuates Tumor Development and the Cancer Cachexia Syndrome in Walker-256 Tumor-Bearing Obese Rats and Improves the Metabolic and Hematological Profiles of These Rats. *Nutr. Cancer* **2021**, *73*, 1175–1192. [CrossRef]
31. Malone, M.H. The pharmacological evaluation of natural products--general and specific approaches to screening ethnopharmaceuticals. *J. Ethnopharmacol.* **1983**, *8*, 127–147. [CrossRef] [PubMed]
32. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Roberts, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–358. [CrossRef]
33. Bidinotto, P.M.; Souza, R.H.S.; Moraes, G. Hepatic glycogen in eight tropical freshwater teleost fish: A procedure for field determinations of micro samples. *Bol. Técnico CEPTA* **1997**, *10*, 53–60. Available online: https://www.icmbio.gov.br/cepta/images/stories/producao_cientifica/hepatic_1997_01.pdf (accessed on 26 August 2024).
34. Harrower, J.R.; Brown, C.H. Blood lactic acid. A micromethod adapted to field collection of microliter samples. *J. Appl. Physiol.* **1972**, *32*, 709–711. [CrossRef] [PubMed]
35. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
36. Spies, J.R. Colorimetric procedures for amino acids. *Methods Enzymol.* **1957**, *3*, 467–477.
37. Gentzkow, C.J.; Masen, J.M. An accurate method for the determination of blood urea nitrogen by direct nesslerization. *J. Biol. Chem.* **1942**, *143*, 531–544. [CrossRef]
38. Misra, H.P.; Fridovich, I. The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **1972**, *247*, 3170–3175. Available online: https://pubmed.ncbi.nlm.nih.gov/4623845/ (accessed on 10 August 2024). [CrossRef]
39. Nelson, D.P.; Kiesow, L.A. Enthalphy of decomposition of hydrogen peroxide by catalase at 25 °C (with molar extinction coefficients of H_2O_2 solution in the UV). *Anal. Biochem.* **1972**, *49*, 474–478. [CrossRef]
40. Habig, W.H.; Pabst, M.J.; Jacoby, W.B. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139. Available online: https://pubmed.ncbi.nlm.nih.gov/4436300/ (accessed on 15 July 2024). [CrossRef]
41. Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of glutathione peroxidase. *J. Lab. Clin. Med.* **1987**, *70*, 158–165. Available online: https://pubmed.ncbi.nlm.nih.gov/6066618/ (accessed on 15 July 2024).
42. Sedlack, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **1968**, *25*, 192–205. [CrossRef] [PubMed]
43. Roe, J.H. Chemical determination of ascorbic, dehydroascorbic, and diketogulonic acids. *Methods Biochem. Anal.* **1954**, *1*, 115–139. [CrossRef] [PubMed]
44. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–309.
45. Colombo, G.; Marco, C.; Garavaglia, M.E.; Giustarini, D.; Rossi, R.; Milzani, A.; Dalle-Donne, I. A step-by-step protocol for assaying protein carbonylation in biological samples. *J. Chromatogr. B* **2016**, *1019*, 178–190. [CrossRef]
46. Kim, D.; Hanzawa, F.; Shimizu, H.; Sun, S.; Umeki, M.; Ikeda, S.; Mochizuki, S.; Oda, H. Delayed feeding of a high-sucrose diet led to increased body weight by affecting the circadian rhythm of body temperature and hepatic lipid-metabolism genes in rats. *J. Nutr. Biochem.* **2023**, *111*, 109185. [CrossRef]
47. Adeoye, B.K.; Oduko, A.O.; Adeoye, A.O.; Ayodele, K.; Uwannah, N.C.; Ani, I.F.; Oyerinde, O.O.; Oyinloye, C. Feeding behaviour, weight gain and blood sugar of male wistar rats fed on a high-calorie diet and vegetables. *Afric. J. Food Agric. Nutr. Dev.* **2022**, *22*, 21127–21145. [CrossRef]
48. Melo, B.F.; Sacramento, J.F.; Ribeiro, M.J.; Prego, C.S.; Correia, M.C.; Coelho, J.C.; Cunha-Guimaraes, J.P.; Rodrigues, T.; Martins, I.B.; Guarino, M.P.; et al. Evaluating the Impact of Different Hypercaloric Diets on Weight Gain, Insulin Resistance, Glucose Intolerance, and its Comorbidities in Rats. *Nutrients* **2019**, *11*, 1197. [CrossRef] [PubMed]
49. Gual-Grau, A.; Guirro, M.; Mayneris-Perxachs, J.; Arola, L.; Boqué, N. Impact of different hypercaloric diets on obesity features in rats: A metagenomics and metabolomics integrative approach. *J. Nutrients Biochem.* **2019**, *71*, 122–131. [CrossRef]
50. Silva, A.Z.; Costa, F.P.L.; Souza, I.L.; Ribeiro, M.C.; Giordani, M.A.; Queiroz, D.A.; Luvizotto, R.A.M.; Nascimento, A.F.; Bomfim, G.F.; Sugizaki, M.M.; et al. Botryosphaeran reduces obesity, hepatic steatosis, dyslipidemia, insulin resistance and glucose intolerance in diet-induced obese rats. *Life Sci.* **2018**, *211*, 147–156. [CrossRef]
51. Ribeiro, M.C.; Silva, A.Z.D.; Giordani, M.A.; Lira, C.; Luviz, M.; Nascimento, A.F.; Queiroz, D.A.; Dekker, R.F.H.; Barbosa-Dekker, A.M.; de Queiroz, E.A.I.F. Extended treatment with (1→3)(1→6)-β-d-glucan (Botryosphaeran) reduces obesity and its comorbidities in high-fat/high-sugar diet-fed rats. *Cell Biochem. Funct.* **2022**, *40*, 773–783. [CrossRef] [PubMed]
52. Carvalho, K.M.; Melo, T.S.; Melo, K.M.; Quinderé, A.L.; Oliveira, F.T.; Viana, A.F.; Nunes, P.I.; Quetz, J.D.; Viana, D.A.; Silva, A.A.; et al. Amyrins from *Protium heptaphyllum* Reduce High-Fat Diet-Induced Obesity in Mice via Modulation of Enzymatic, Hormonal and Inflammatory Responses. *Plant Medica* **2017**, *83*, 285–291. [CrossRef] [PubMed]
53. Carvalho, K.M.; Marinho Filho, J.D.; Melo, T.S.; Araújo, A.J.; Quetz, J.d.S.; da Cunha, M.D.P.S.S.; de Melo, K.M.; Silva, A.A.d.C.A.d.; Tomé, A.R.; Havt, A.; et al. The Resin from *Protium heptaphyllum* Prevents High-Fat Diet-Induced Obesity in Mice: Scientific Evidence and Potential Mechanisms. *Evid. Based Complement. Altern. Med.* **2015**, *2015*, 106157. [CrossRef] [PubMed]

54. Santos, F.A.; Frota, J.T.; Arruda, B.R.; Melo, T.S.; Silva, A.A.; Brito, G.A.; Chaves, M.H.; Rao, V.S. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis.* **2012**, *11*, 98. [CrossRef]
55. Patias, N.S.; Sinhorin, V.D.G.; Sinhorin, A.P. Potential antioxidants and other biological activities of *Protium heptaphyllum* (Aubl.). March: Mini-Review. *Nat. Prod. J.* **2023**, *13*, e090223213549. [CrossRef]
56. Rufino, A.T.; Costa, V.M.; Carvalho, F.; Fernandes, E. Flavonoids as antiobesity agents: A review. *Med. Res. Rev.* **2021**, *41*, 556–585. [CrossRef]
57. Espírito-Santo, D.A.; Cordeiro, G.S.; Santos, L.S.; Silva, R.T.; Pereira, M.U.; Matos, R.J.B.; Boaventura, G.T.; Barreto-Medeiros, J.M. Cardioprotective effect of the quercetin on cardiovascular remodeling and atherosclerosis in rodents fed a high-fat diet: A systematic review. *Chem. Biol. Interact.* **2023**, *384*, 110700. [CrossRef]
58. Smout, D.; Joergensen, H.; Meijers, B.; Dejongh, S.; Haarhaus, M.; Cavalier, E.; Craenenbroeck, A.H.V.; Evenepoel, P. #4346 Serum nonskeletal alkaline phosphatase as a marker of metabolic endotoxinemia and predictor of adverse outcome in patients with ckd. *Nephrol. Dial. Transplant.* **2023**, *38*, gfad063c_4346. [CrossRef]
59. Kohli, R.; Harris, D.C.; Whitington, P.F. Relative elevations of serum alanine and aspartate aminotransferase in muscular dystrophy. *J. Pediatr. Gastroenterol. Nutr.* **2005**, *41*, 121–124. [CrossRef]
60. Nelson, D.L.; Cox, M.M. *Princípios de Bioquímica de Lehninger*, 6th ed.; Artmed: São Paulo, Brazil, 2014.
61. Oliveira, F.A.; Vieira-Júnior, G.M.; Chaves, M.H.; Almeida, F.R.; Florêncio, M.G.; Lima, R.C.; Silva, R.M.; Santos, F.A.; Rao, V.S. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. *Pharmacol. Res.* **2004**, *49*, 105–111. [CrossRef]
62. Huidobro, E.J.P.; Tagle, R.; Guzmán, A.M. Creatinina y su uso para la estimación de la velocidad de filtración glomerular [Estimation of glomerular filtration rate with creatinine]. *Rev. Med. Chil.* **2018**, *146*, 344–350. [CrossRef] [PubMed]
63. Marseglia, L.; Manti, S.; D'Angelo, G.; Nicotera, A.; Parisi, E.; Di Rosa, G.; Gitto, E.; Arrigo, T. Oxidative stress in obesity: A critical component in human diseases. *Int. J. Mol. Sci.* **2014**, *16*, 378–400. [CrossRef]
64. Manna, P.; Jain, S.K. Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies. *Metab. Syndr. Relat. Disord.* **2015**, *13*, 423–444. [CrossRef] [PubMed]
65. Masenga, S.K.; Kabwe, L.S.; Chakulya, M.; Kirabo, A. Mechanisms of Oxidative Stress in Metabolic Syndrome. *Int. J. Mol. Sci.* **2023**, *24*, 7898. [CrossRef]
66. Sinhorin, V.D.G.; Braga, A.J.L.; Rosa, A.P.; Ferneda, J.M.A.; Moura, F.R.; Ferreira, C.M.; Abreu, J.S.; Hoshiba, M.A.; Fontinhas Netto, G.V. Protective effect of hydroxy-selenomethionine supplementation in the diet of tambaqui (*Colossoma macropomum*) subjected to transportation stress. *Acta Amaz.* **2024**, *54*, e54af23091. [CrossRef]
67. Mahboob, A.; Samuel, S.M.; Mohamed, A.; Wani, M.Y.; Ghorbel, S.; Miled, N.; Büsselberg, D.; Chaari, A. Role of flavonoids in controlling obesity: Molecular targets and mechanisms. *Front. Nutr.* **2023**, *10*, 1177897. [CrossRef]
68. Raut, S.K.; Khullar, M. Oxidative stress in metabolic diseases: Current scenario and therapeutic relevance. *Mol. Cell. Biochem.* **2023**, *478*, 185–196. [CrossRef]
69. Cavaliere, G.; Catapano, A.; Trinchese, G.; Cimmino, F.; Menale, C.; Petrella, L.; Mollica, M.P. Crosstalk between Adipose Tissue and Hepatic Mitochondria in the Development of the Inflammation and Liver Injury during Ageing in High-Fat Diet Fed Rats. *Int. J. Mol. Sci.* **2023**, *24*, 2967. [CrossRef]
70. Sandoval, V.; Sanz-Lamora, H.; Arias, G.; Marrero, P.F.; Haro, D.; Relat, J. Metabolic Impact of Flavonoids Consumption in Obesity: From Central to Peripheral. *Nutrients* **2020**, *12*, 2393. [CrossRef]
71. Siani, A.C.; Ramos, M.F.; Menezes-de-Lima, O.; Ribeiro-dos-Santos, R.; Fernadez-Ferreira, E.; Soares, R.O.; Rosas, E.C.; Susunaga, G.S.; Guimarães, A.C.; Zoghbi, M.G.; et al. Evaluation of anti-inflammatory-related activity of essential oils from the leaves and resin of species of *Protium*. *J. Ethnopharmacol.* **1999**, *66*, 57–69. [CrossRef]
72. Van Der Heijden, R.A.; Morrison, M.C.; Sheedfar, F.; Mulder, P.; Schreurs, M.; Hommelberg, P.P.; Hofker, M.H.; Schalkwijk, C.; Kleemann, R.; Tietge, U.J.; et al. Effects of Anthocyanin and Flavanol Compounds on Lipid Metabolism and Adipose Tissue Associated Systemic Inflammation in Diet-Induced Obesity. *Mediat. Inflamm.* **2016**, *2016*, 2042107. [CrossRef] [PubMed]
73. Kaushik, S.; Kaur, J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. *Clin. Chim. Acta* **2003**, *333*, 69–77. [CrossRef] [PubMed]
74. Kostopoulou, E.; Varemmenou, A.; Kalaitzopoulou, E.; Papadea, P.; Skipitari, M.; Rojas Gil, A.P.; Spiliotis, B.E.; Fouzas, S.; Georgiou, C.D. New Clinical Markers of Oxidized Lipid-Associated Protein Damage in Children and Adolescents with Obesity. *Children* **2024**, *11*, 314. [CrossRef]
75. Zhang, M.; Yuan, W.G.; Li, C.; Liu, X.; Ma, Z.L.; Xian, Y.F.; Chen, Z.S.; Wang, C.X.; Li, L.; Xu, Z.; et al. Antioxidants Accelerate Hepatocellular Carcinoma Initiation and Progression by Inhibiting Gst-Pi-Mapk Axis. *Cell Biosci.* **2023**. [CrossRef]
76. Kitamura, Y.; Oikawa, S.; Chang, J.; Mori, Y.; Ichihara, G.; Ichihara, S. Carbonylated Proteins as Key Regulators in the Progression of Metabolic Syndrome. *Antioxidants* **2023**, *12*, 844. [CrossRef]
77. Oliveira, F.A.; Chaves, M.H.; Almeida, F.R.; Lima, R.C.; Silva, R.M.; Maia, J.L.; Brito, G.A.A.; Santos, F.A.; Rao, V.S. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.* **2005**, *98*, 103–108. [CrossRef]

78. Awodele, O.; Yemitan, O.; Ise, P.U.; Ikumawoyi, V.O. Modulatory potentials of aqueous leaf and unripe fruit extracts of *Carica papaya* Linn. (*Caricaceae*) against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats. *J. Intercult. Ethnopharmacol.* **2016**, *5*, 27–35. [CrossRef]
79. Sarkar, S.; Das, M.; Nanjappan, S. Exploration of the Pharmacological Mechanism of Herbal Medicines against Hepatic Disorder: A Systematic Review and Network Pharmacological Analysis. *Pharmacol. Res. Nat. Prod.* **2024**, *3*, 100048. [CrossRef]
80. Oliveira, A.; Augustin, S.; Benlloch, S.; Ampuero, J.; Suárez-Pérez, J.A.; Armesto, S.; Vilarrasa, E.; Belinchón-Romero, I.; Herranz, P.; Crespo, J.; et al. The Essential Role of IL-17 as the Pathogenetic Link between Psoriasis and Metabolic-Associated Fatty Liver Disease. *Life* **2023**, *13*, 419. [CrossRef]
81. Li, N.; Yamamoto, G.; Fuji, H.; Kisseleva, T. Interleukin-17 in Liver Disease Pathogenesis. *Semin. Liver Dis.* **2021**, *41*, 507–515. [CrossRef]
82. Barrón, J.C.S.; González, C.C.; Parrilla, E.Á.; De la Rosa, L.A. Nanoparticle-Mediated Delivery of Flavonoids: Impact on Proinflammatory Cytokine Production: A Systematic Review. *Biomolecules* **2023**, *3*, 1158.

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CAPÍTULO 4

Study of Liposomes Containing Extract from the Leaves of *Protium heptaphyllum* (Aubl.) March in Animals Submitted to a Mutagenic Model Induced by Cyclophosphamide

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Study of Liposomes Containing Extract from the Leaves of *Protium heptaphyllum* (Aubl.) March in Animals Submitted to a Mutagenic Model Induced by Cyclophosphamide

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Simple Summary: In this study, the protective effect of *Protium heptaphyllum* (*P. heptaphyllum*), a plant with a high flavonoid content, was analyzed against damage caused by cyclophosphamide (CPA), a chemotherapy drug known for its adverse effects. Using liposomes to transport the plant extract, an experiment was carried out with male Swiss mice that received the extract for 14 days before CPA. The results demonstrated that *P. heptaphyllum* liposomes reduced DNA damage and oxidative stress, evidenced by the increase in antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). There were also improvements in liver and kidney indicators, suggesting organ protection. In summary, these results highlight the potential of *P. heptaphyllum* liposomes as a natural chemical protection agent that can help reduce the side effects of cancer treatment, while providing antioxidant benefits.

Abstract: Cyclophosphamide (CPA) is an alkylating agent used as a chemotherapy agent in the treatment of cancer, but it has immunosuppressive effects. *Protium heptaphyllum* (*P. heptaphyllum*) is a plant rich in triterpenes and flavonoids, with some bioactive and therapeutic properties presented in the literature. Thus, the present study aimed to investigate the chemoprotective potential of *P. heptaphyllum* extract inserted into liposomes against oxidative damage chemically induced by CPA. Male Swiss mice received 1.5 mg/kg of *P. heptaphyllum* liposomes as a pre-treatment for 14 consecutive days (via gavage) and 100 mg/kg of CPA in a single dose (via intraperitoneal) on the 15th day. After the experimental period, blood and organ samples were collected for histopathological and biochemical analyses, including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione S-transferase (GST), reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), ascorbic acid (ASA), carbonyl protein, cytokine measurement, and micronucleus testing. The results showed that liposomes containing *P. heptaphyllum* extract have an antimutagenic effect against damage induced to DNA by CPA, and that they also protect against oxidative stress, as verified by the increase in the antioxidant enzymes SOD and GPx. The improvement in alkaline phosphatase and creatinine markers suggests a beneficial effect on the liver and kidneys, respectively. However, the depletion of GSH in the liver and brain suggests the use of antioxidants for the

metabolism of molecules generated in these tissues. In general, these data show good prospects for the use of *P. heptaphyllum* liposomes as a cancer chemoprotective agent, as well as possible antioxidant action, conceivably attributed to the flavonoids present in the plant extract.

Keywords: oxidative stress; antimutagenic; cancer; flavonoid; quercetin

1. Introduction

Cyclophosphamide (CPA) is a drug extensively used as an alkylating agent in the treatment of several malignant neoplasms, including breast cancer, multiple myeloma, kidney disease, rheumatoid arthritis, juvenile dermatomyositis, systemic sclerosis, interstitial lung disease, lupus vasculopathy, systemic vasculitis, and refractory treatment of thrombocytopenic purpura [1,2]. CPA can be administered orally or intravenously, with oral dosing administered daily (every 24 h) and in pulses, adjusting the dose according to hematologic and renal toxicity. Adverse reactions to CPA include bone marrow suppression, susceptibility to infections, sterility, amenorrhea, nephrotoxicity, and cystitis, as well as cardiovascular complications such as sinus bradycardia, pericarditis, myocarditis, and heart failure [1–4].

The search for new drugs that help treat pathologies while exerting fewer side effects is essential to improve patients' quality of life and to advance medicine. The wealth of substances derived from plants has aroused great interest in this field among scientific community as a promising source for the development of new drugs. The search for innovative medicinal agents, especially those derived from natural sources, is driven by the presence of several molecules in the secondary metabolism of plants [5–7]. Many drugs originate directly or indirectly in plant compounds, and plants harbor a variety of phytoconstituents, each with unique and distinct properties [8].

Protium heptaphyllum (*P. heptaphyllum*) is a plant commonly known as almacega, almíscar or breu branco, and it is typically found in the Amazon [9]. Its leaves and resin are used in folk medicine due to their stimulating, anti-inflammatory, and healing properties [10,11]. It derives from the Burceraceae family, comprising 18 genera and more than 700 species divided into three tribes, with the genus Protium (tribe Protieae) being the main member of the family [12,13].

The resins, oils, and leaves of the genus Protium are extremely rich in terpenes and polyphenols, such as flavonoids [5,12,14]. Phenolic compounds, including flavonoids, are known as free radical scavengers and are highly efficient in preventing autoxidation. Studies indicate that flavonoids can reduce cellular stress, which includes neuroinflammation, oxidative stress, proteotoxicity, and endoplasmic reticulum stress [15]. Antioxidants, in addition to providing several beneficial effects, can inhibit or delay the emergence of tumor cells, delay aging, and prevent other cellular damage resulting from redox imbalance [16–18].

Liposome nanotechnology is one of the new, innovative therapeutic approaches based on plants. This approach improves the solubility, stability, and specific targeting of active substances, overcoming absorption and bioavailability challenges [19]. By encapsulating an active substance, liposomes offer controlled release, minimize side effects, and maximize therapeutic efficacy, presenting a promising frontier for the optimization of phytotherapeutic treatments in contemporary medicine [19,20]. Thus, herbal medicines conjugated using new technologies, such as using liposomes, can aid in treatment by offering effective, accessible solutions, with fewer side effects, for various pathologies due to the variety of chemical compounds, such as saponins, flavonoids, and catechins, present in plant species, highlighting the need for studies on the biological activity and toxicity of these plants to encourage investments from the pharmaceutical industry [16,17,21]. Therefore, this research sought to investigate the preventive chemoprotective effects of the ethyl acetate

fraction of *P. heptaphyllum* extract inserted in liposomes against the oxidative processes caused by CPA.

2. Material and Methods

2.1. Preparation of Extract and Development of Liposomes

The work was developed at the Integrated Chemical Sciences Research Laboratories (LIPEQ), located at the Federal University of Mato Grosso, Campus of Sinop. The exsiccata is registered in the collection of the Centro-Norte Mato-Grossense Herbarium (CNMT) of the Federal University of Mato Grosso, located at the Campus of Sinop, under number 625.

The leaves of the species *P. heptaphyllum* were collected and selected, dried, crushed into powder, and subsequently macerated with ethanol for 7 days. Next, 1.05223 kg of leaf powder were mixed with 4 L of ethanol. After the maceration period, chlorophyll was removed using activated charcoal (69.57 g). Then, the extract underwent the solvent rotary evaporation process, followed by lyophilization to obtain the crude ethanolic extract (CEE), and was finally subjected to functional group identification tests. A portion of the CEE was fractionated through a silica gel chromatographic column, using a solvent gradient of increasing polarity; additional details can be found in the work of Patias et al. [14]. After fractionation, the choice was made to employ the ethyl acetate fraction (EAF) of *P. heptaphyllum* in the development of liposomes (LP) due to its high concentration of flavonoids.

The liposomes were developed using the reverse-phase evaporation method, followed by lipid film extrusion, as described by Hua and Wu [22].

The liposomes were characterized physicochemically by evaluating the active compound content and average micelle diameter using a Mastersizer® 2000 device (Malvern, England); the polydispersity index (PDI) using photon brightness spectroscopy analysis (Zetasizer® NanoZS model ZEN 3600, Malvern Instruments, (Malvern, England); the zeta potential by electrophoretic mobility; pH in a potentiometer (Denver® Instrument VB-10, New York, NY, USA); as well as the dosage of quercetin in the nanosystem. The results obtained from the characterization of *P. heptaphyllum* liposomes show that the technique used allowed the formation of particles on the nanometric scale. Micelles with small size (232 ± 1.70) and low polydispersity index (0.21 ± 0.01) characterize a system with low size distribution. The zeta potential value demonstrates the surface charge potential (-20.07 ± 1.10), which is a fundamental parameter to predict the stability of liposomes. Zeta potential values close to -20 mV (as occurs with the *P. heptaphyllum* liposomes in the present study) can be considered satisfactory to maintain the stability of the system [18]. The charges found in the developed liposomal formulations follow the standard for formulations based on DPPC, in which the occurrence of a negative charge is predominant among the samples.

2.2. In Vivo Tests

Initially, the Malone hippocratic test was performed to construct a dose curve for liposomes containing *P. heptaphyllum* extract at concentrations of 0.5, 1.0, 1.5, 2.5, and 5 mg/kg, using three animals for each concentration. This procedure aimed to analyze the potential acute toxicity of the doses used, as described by Malone [23]. Based on the results obtained, a specific dose of 1.5 mg/kg was then chosen to be administered to the animals.

2.2.1. Animals and Experimental Design

Male Swiss mice weighing an average of $35 \text{ g} \pm 3 \text{ g}$ were used for the study. All steps for authorization to work with animals were approved by the Guidelines of the Ethics Committee for the Use of Animals of the Federal University of Mato Grosso, No. 23108.030996/2022-07. A total of eight animals per group were employed for a treatment period of 14 days.

Throughout the experimental period, the animals were kept in polypropylene boxes with a controlled temperature of $22 \pm 2\ ^{\circ}\text{C}$ and a 12 h light–dark cycle, with free access to water

and food. The animals were distributed into four experimental groups according to whether or not they were treated with liposomes containing *P. heptaphyllum* leaf extract, as follows:

Group C: Control; CPA Group: cyclophosphamide; LP Group: liposome with *P. heptaphyllum* extract; LP + CPA Group: liposome with *P. heptaphyllum* extract + cyclophosphamide.

The acclimatization period was 2 weeks. The groups treated with liposomes containing the EAF extract (LP, LP + CPA) received a dose of 1.5 mg/kg orally (v.o.), once a day, for 14 days. The Control groups (C and CPA) received water solution (H_2O) in the same volume, v.o., also for 14 days.

CPA was supplied by Baxter®, diluted in 0.9% saline solution, and administered to the animals intraperitoneally (i.p.) on the 15th day at a concentration of 100 mg/kg (0.1 mL/10 g body weight) [24].

After the stipulated period, the animals were fasted for 8 h, and at the end of the 16th day, blood was collected by cardiac puncture under anesthesia (Chlortamine® (Ketamine, 50 mg/kg) and Rompun® (Xylazine, 20 mg/kg)); the animals were euthanized by cervical dislocation. Samples of the liver, kidneys, heart, and brain were collected by dissection, washed with isotonic saline solution, and weighed to determine absolute (g) and relative (g/100 g body weight) weight. In addition, the weight of the mice (g) was assessed; part of the tissue samples were stored frozen at −80 °C and part fixed in 10% phosphate-buffered formalin for subsequent analysis. Plasma was obtained from whole blood after centrifugation (4000 rpm, 10 min; Nova Técnica, NT-835, Piracicaba, Brazil).

2.2.2. Analysis of Anthropometric Measurements

Body weight, as well as daily water and food consumption, were assessed at the beginning and end of the experiment. To analyze consumption, 500 g of food were placed in the box, and the uneaten food was weighed every two days. The difference represented the amount of food consumed by the mice in the box, expressed in grams. The same protocol was used to analyze water consumption, but in mL measurements, where 500 mL of water were offered. This analysis was performed throughout the treatment.

2.2.3. Micronucleus Test

The preparation and collection of bone marrow erythrocyte slides for evaluation of micronucleus (MN) frequency followed the methodology proposed by MacGregor et al. [25]. For each animal, duplicate smears were created and stained to differentiate polychromatic erythrocytes (PCE) from normochromic erythrocytes (NCE). From these prepared slides, 1000 cells per animal were analyzed under a light microscope (Kasvi/Olen, K55-BA, Pinhais, PR, Brazil), with 1000× magnification (immersion). The material was analyzed in a blind test, and the slides were decoded at the end of the analyses.

2.2.4. Biochemical Analyses of Liver, Kidney, Brain, Heart, and Plasma

The biochemical parameters (glucose, aspartate aminotransferase—AST, alkaline phosphatase—ALP, total cholesterol, triglycerides, and creatinine) of blood plasma were analyzed using commercial kits (Labtest®, Diagnóstico S. A., Minas Gerais, Brazil).

The enzymatic antioxidant activity of CAT (catalase) was determined according to the methods of Nelson and Kiesow [26], SOD (superoxide dismutase) according to the suggestions of Misra and Fridovich [27], GST (glutathione S-transferase) according to the work of Habig et al. [28], and GPx (glutathione peroxidase) was measured according to the techniques of Paglia and Valentine [29]. The dosage of the non-enzymatic antioxidants GSH (reduced glutathione) was evaluated according to the methods of Sedlack and Lindsay [30], and ascorbic acid (ASA) was measured according to the suggestions of Roe [31]. The indirect markers of oxidative damage evaluated included TBARS (thiobarbituric acid reactive substances) and carbonyl (carbonylated proteins), according to the technique described by Buege and Aust and Colombo et al., respectively [32,33]. The protein content of the tissues was determined according to the work of Bradford [34]. All analyses were performed using a UV–Vis spectrophotometer (Varian Cary 50 Scan, Northfield, MI, USA).

2.2.5. Histological Analysis

Liver and kidney fragments were used for histopathological analysis. These were fixed in 10% phosphate-buffered formalin for 48 h and immersed in 70% alcohol. The tissue fragments were cleaved to a thickness of 0.4 cm, placed in cassettes, and subjected to the dehydration and clearing process in an automatic histotechnician (Lupitec, PT 05 TS, São Carlos, SP, Brazil). They were later embedded in paraffin and cut to a thickness of 3 µm using a semi-automatic microtome (Leica Biosysthems RM2245, Nussloch, Germany). The slides were stained with hematoxylin and eosin and evaluated under an optical microscope (Nikon eclipse 80I, Tokyo, Japan). Histological evaluation was carried out along the entire length of the slide fragment, with magnification objectives of 10, 40, and 60 times. A trained technician evaluated the occurrence of circulatory, degenerative, proliferative, necrotic, and inflammatory changes. The changes were altered in terms of distribution and intensity, according to the methods of Barros et al. (2021) [35].

2.2.6. Immunological Analysis by ELISA

To perform immunological analyses of cytokines, such as tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 17 (IL-17), and interleukin 1β (IL-1β), the enzyme-linked immunosorbent assay (ELISA) methodology was employed using commercial kits from DIY Duoset Elisa®, according to the instructions provided by the manufacturer.

2.3. Data Analysis

The data were initially subjected to the Kolmogorov–Smirnov normality test. Then, a two-way analysis of variance (ANOVA) was performed, followed by the Tukey–Kramer multiple comparison test to assess the means when more than two groups were present. In cases where the results did not meet the normality criteria, the Kruskal–Wallis test was used, followed by Dunn's post-test. The acceptable significance level adopted was $p < 0.05$. The results were presented as mean ± standard deviation or median and total range, according to the protection obtained. Graphs were created and statistical analysis was performed using Graph Pad Prism 8.0 statistical software.

The frequency of micronucleated polychromatic erythrocytes (MCPEs) in the different experimental groups was compared using the Chi-squared test [36]. The percentage of damage reduction (decrease in the mean frequency of micronucleated cells) of the extracts that showed antimutagenic activity were calculated according to the method of Waters et al. [37], using the following formula:

$$\%\ reduction = \frac{Frequency\ of\ PCEMs\ in\ a - Frequency\ of\ PCEMs\ in\ b}{Frequency\ of\ PCEMs\ in\ a - Frequency\ of\ PCEMs\ in\ c} \times 100 \quad (1)$$

where:

a = group treated with CPA;
b = group treated with LP + CPA;
c = group treated with 0.9% NaCl (control).

3. Results

The measurements of initial body weight, final body weight, and feed and water consumption did not show statistically significant differences between the groups analyzed, as shown in Table 1. However, there were changes in the weights of the organs investigated, such as the liver and heart. In the liver, the weight increased in the CPA group when compared to that in the C group, and the LP + CPA group showed a reduction in weight when compared to that of the LP and CPA groups. For the heart, the CPA group showed an increase in weight compared to that of the C group, and in the LP + CPA group, there was a decrease when compared to the results for the CPA group, as indicated in Table 1. The other organs investigated did not show statistically significant changes.

Table 1. Body and organ weights and food and water intake of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

Parameters	Control (C)	CPA	LP	LP + CPA
Initial body weight (g)	41.75 ± 5.23	41.12 ± 2.74	41.37 ± 2.92	39.75 ± 3.73
Final body weight (g)	44.12 ± 5.54	42.50 ± 2.13	41.12 ± 2.90	42.37 ± 3.70
Feed consumption (g/day/mouse)	44.33 ± 9.64	47.00 ± 14.53	45.00 ± 10.23	47.83 ± 13.12
Water intake (mL/day/mouse)	28.73 ± 4.21	41.03 ± 13.04	31.20 ± 6.83	29.46 ± 6.89
Liver (g)	1.72 ± 0.13	2.06 ± 0.11 *	1.81 ± 0.12	1.63 ± 0.11 **,#
Kidney (g)	0.51 ± 0.05	0.56 ± 0.05	0.56 ± 0.05	0.51 ± 0.03
Heart (g)	0.19 ± 0.008	0.22 ± 0.01 *	0.21 ± 0.02	0.19 ± 0.02 #
Brain (g)	0.43 ± 0.05	0.42 ± 0.04	0.43 ± 0.06	0.43 ± 0.08

Results are presented as mean ± standard deviation using ANOVA (two-way), followed by Tukey's post-hoc test. * $p < 0.05$ vs. C; ** $p < 0.05$ vs. LP; # $p < 0.05$ vs. CPA; (n = 8).

Plasma glucose, cholesterol and aspartate aminotransferase (AST) enzyme activity levels did not show statistically significant changes in the groups analyzed. There was a decrease in alkaline phosphatase (ALP) activity in the LP + CPA group compared to that in group C. In addition, creatinine levels were reduced in the CPA, LP, and LP + CPA groups compared to those noted in group C. As for triglycerides, a decrease was observed in the CPA group compared to the levels in group C, as shown in Table 2.

Table 2. Analysis of plasma parameters of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

Parameters	Control (C)	CPA	LP	LP + CPA
Glucose (mg/dL)	229.25 ± 57.17	224.62 ± 44.51	185.12 ± 43.79	251.75 ± 53.54
AST (U/L)	154.87 ± 42.00	185.50 ± 150.00	182.25 ± 97.00	186.12 ± 181.00
ALP (U/L)	112.62 ± 25.39	100.12 ± 19.65	96.62 ± 18.57	76.87 ± 7.84 *
Creatinine (mg/dL)	3.18 ± 0.66	2.13 ± 1.03 *	1.31 ± 0.46 *	1.45 ± 0.50 *
Cholesterol (mg/dL)	85.87 ± 14.24	79.80 ± 17.72	102.88 ± 32.83	95.30 ± 25.04
Triglycerides (mg/dL)	145.25 ± 16.62	126.00 ± 3.84 *	128.12 ± 16.27	136.12 ± 5.41

Results are presented as mean ± standard deviation using ANOVA (two-way), followed by Tukey's post-hoc test. For AST analysis, a Kruskal–Wallis test, followed by Dunn's post-hoc test, was used, and values were expressed as median and total range. * $p < 0.05$ vs. C. (AST: aspartate aminotransferase; ALP: alkaline phosphatase); (n = 8).

Table 3 shows the frequency of micronucleated polychromatic erythrocytes (PCEMN) after the pretreatment of mice with liposomes containing *P. heptaphyllum* extract on chemically induced damage by CPA. The group treated with LP + CPA showed a significant reduction ($p \leq 0.05$) in the frequency of micronuclei when compared with that of the positive control group, showing a chemoprotective effect of the extract and thus, the possibility of producing benefits related to the prevention of DNA damage as an antimutagenic agent. On the other hand, the group treated only with the liposome containing the extract did not show a mutagenic effect when compared with the negative control group.

We did not observe statistical changes between the groups studied for cytokine levels in the liver and kidney tissue (Table 4).

In the liver, a decrease in the activity of the catalase enzyme was observed in the LP + CPA group compared to that in the C groups and the CPA group (Figure 1B). On the other hand, the GST enzyme showed an increase in activity in the LP + CPA group compared to the LP group (Figure 1C). As for the SOD and GPx enzymes, no statistically significant changes were observed (Figures 1A and 1D, respectively). In the renal tissue, an increase in the activity of the SOD enzyme was observed in the CPA group compared to that in the C group (Figure 1E). No significant differences were observed in the activity of the catalase enzyme between the groups studied (Figure 1F), while the GST enzyme showed decreased activity in all treated groups, as illustrated in Figure 1G. The GPx enzyme increased its activity in the LP + CPA group when compared to that in the CPA group (Figure 1H).

Table 3. Frequency of micronucleated polychromatic erythrocytes (PCEMN) in bone marrow of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

	PCEMNs			
Treatment	**Number of PCEs Analyzed**	**MN**	**%**	**% Reduction**
Control (Water + NaCl 0.9%)	8000	290	3.62	
CPA (Water + CPA)	8000	535	6.69	
LP (LP + NaCl 0.9%)	8000	264	3.30	
LP + CPA (LP + CPA)	8000	470 *	5.81	26%

* $p < 0.05$ in comparison with the CPA group, according to the Chi-squared test; (n = 8).

Table 4. Analysis of cytokines in liver and kidney tissue of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

	Parameters	**Control (C)**	**CPA**	**LP**	**LP + CPA**
Liver	TNF-α (pg/mL)	0.72 ± 0.26	0.76 ± 0.24	0.83 ± 0.25	0.97 ± 0.15
	IFN-γ (pg/mL)	1.17 ± 0.19	1.20 ± 0.12	1.24 ± 0.11	1.27 ± 0.07
	IL-6 (pg/mL)	1.05 ± 0.18	1.12 ± 0.14	1.13 ± 0.10	1.15 ± 0.04
	IL-10 (pg/mL)	1.15 ± 0.26	1.27 ± 0.17	1.28 ± 0.10	1.37 ± 0.08
	IL-17 (pg/mL)	0.97 ± 0.22	1.11 ± 0.24	1.13 ± 0.17	1.18 ± 0.11
	IL-β (pg/mL)	0.77 ± 0.22	0.89 ± 0.11	0.97 ± 0.17	1.01 ± 0.13
Kidney	TNF-α (pg/mL)	0.96 ± 0.21	1.15 ± 0.32	0.93 ± 0.30	0.94 ± 0.21
	IFN-γ (pg/mL)	1.30 ± 0.14	1.46 ± 0.09	1.34 ± 0.20	1.42 ± 0.19
	IL-6 (pg/mL)	1.21 ± 0.18	1.38 ± 0.20	1.28 ± 0.25	1.24 ± 0.23
	IL-10 (pg/mL)	1.24 ± 0.18	1.39 ± 0.11	1.31 ± 0.27	1.28 ± 0.27
	IL-17 (pg/mL)	1.25 ± 0.13	1.16 ± 0.17	1.09 ± 0.23	1.17 ± 0.13
	IL-β (pg/mL)	1.00 ± 0.23	1.16 ± 0.36	1.23 ± 0.28	1.12 ± 0.26

Results are presented as mean ± standard deviation. ANOVA (two-way) followed by Tukey's post-hoc test; (n = 8). For statistical analysis, data were transformed into sqrt.

GSH levels in the liver tissue decreased in the CPA, LP, and LP + CPA groups compared to those found in group C. In addition, carbonyl protein levels (carbonyl) increased in the CPA group compared to those in group C (Table 5). The other parameters analyzed in the liver tissue (ASA and TBARS) did not show significant changes in their results when compared to those of the control group (C) (Table 5). No statistical differences were observed in redox state markers such as GSH, ASA, and carbonyl in the renal tissue of the animals analyzed. TBARS showed a significant difference, with a decrease in the LP group compared to the levels in group C (Table 4).

Table 5. Analysis of redox status parameters in the liver and kidney tissue of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

	Parameters	**Control (C)**	**CPA**	**LP**	**LP + CPA**
Liver	GSH (µmol de GSH/mg protein)	3.65 ± 1.15	1.96 ± 0.49 *	2.60 ± 0.53 *	2.36 ± 0.64 *
	ASA (µmol ASA/g tissue)	3.05 ± 0.48	3.33 ± 1.04	2.73 ± 0.49	3.28 ± 0.97
	TBARS (nmol MDA/mg protein)	0.71 ± 0.20	0.89 ± 0.19	0.56 ± 0.18	0.66 ± 0.42
	Carbonyl (nmol carbonyl/mg protein)	13.60 ± 2.98	19.06 ± 3.76 *	15.30 ± 4.73	17.16 ± 3.55
Kidney	GSH (µmol de GSH/mg protein)	2.82 ± 3.65	3.53 ± 5.54	1.68 ± 4.08	2.35 ± 5.68
	ASA (µmol ASA/g tissue)	1.40 ± 0.22	1.68 ± 0.21	1.38 ± 0.35	1.42 ± 0.33
	TBARS (nmol MDA/mg protein)	0.10 ± 0.15	0.09 ± 0.05	0.07 ± 0.05 *	0.08 ± 0.07
	Carbonyl (nmol carbonyl/mg protein)	5.35 ± 2.25	5.99 ± 1.41	6.08 ± 2.02	4.89 ± 2.27

The results are presented as mean ± standard deviation. ANOVA (two-way), followed by Tukey's post-hoc test. For analysis of GSH and TBARS of the kidney, the Kruskal–Wallis test, followed by Dunn's post-hoc test, was used, and the values were expressed as median and total range. * $p < 0.05$ vs. C. (GSH: reduced glutathione; ASA: ascorbic acid; TBARS: thiobarbituric acid reactive substances; Carbonyl: carbonylated proteins); (n = 8).

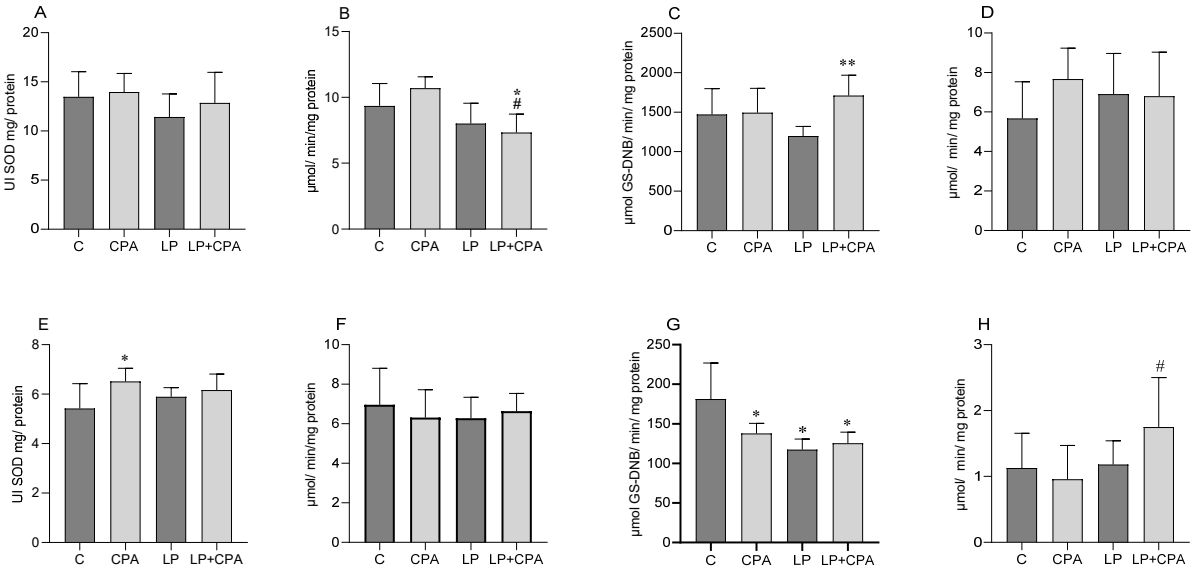

Figure 1. Analysis of enzymatic activities of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide). SOD: superoxide dismutase (**A**), CAT: catalase (**B**), GST: glutathione S-transferase (**C**), e GP_X: glutathione peroxidase (**D**) in the liver; SOD: superoxide dismutase (**E**), CAT: catalase (**F**), GST: glutathione S-transferase (**G**), e GPx: glutathione peroxidase (**H**) in the kidney. The results are presented as mean ± standard deviation. ANOVA (two-way), followed by Tukey's post-hoc test. * $p < 0.05$ vs. C; ** $p < 0.05$ vs. LP; # $p < 0.05$ vs. CPA; (n = 8).

We did not observe any significant changes in the histological analyses.

The activity of the SOD enzyme in brain tissue increased in the LP group compared to that observed in the control group. However, in the LP + CPA group, a reduction in this activity was observed in relation to the LP group (Figure 2A). Regarding the activity of the CAT and GST enzymes, no statistically significant changes were detected (Figure 2B,C).

In cardiac tissue, a significant increase in the activity of the SOD enzyme was observed in the group treated with LP + CPA compared to the group that received only CPA (Figure 2D). Regarding the analysis of the activities of catalase and GST, no changes were identified between the groups analyzed (Figure 2E,F).

We observed a reduction in GSH levels in the brain tissue in the LP + CPA groups compared to the levels in the CPA group. Regarding the levels of ASA, TBARS, and carbonyl, no statistically significant differences were identified between the groups investigated (Table 6).

Table 6. Analysis of redox status parameters in brain and heart tissue of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide).

	Parameters	Control (C)	CPA	LP	LP + CPA
Brain	GSH (µmol de GSH/mg protein)	2.60 ± 3.42	3.67 ± 3.35	1.65 ± 1.30	1.93 ± 1.90 #
	ASA (µmol ASA/g tissue)	3.46 ± 0.34	3.31 ± 0.32	3.54 ± 0.40	3.13 ± 0.26
	TBARS (nmol MDA/mg protein)	4.57 ± 1.40	5.15 ± 0.72	4.41 ± 0.66	5.00 ± 0.87
	Carbonyl (nmol carbonyl mg protein)	18.45 ± 4.25	17.37 ± 3.40	24.30 ± 6.87	23.50 ± 3.08
Heart	GSH (µmol de GSH/mg protein)	1.05 ± 0.34	0.91 ± 0.32	1.81 ± 0.65	1.38 ± 0.79
	ASA (µmol ASA/g tissue)	1.77 ± 0.39	1.30 ± 0.31 *	1.52 ± 0.35	1.46 ± 0.28
	TBARS (nmol MDA/mg protein)	0.67 ± 0.21	0.63 ± 0.10	0.55 ± 0.18	0.78 ± 0.13
	Carbonyl (nmol carbonyl/mg protein)	23.56 ± 11.48	21.79 ± 18.93	25.81 ± 16.33	36.15 ± 31.28

The results are presented as mean ± standard deviation. ANOVA (two-way), followed by Tukey's post-hoc test. For analysis of GSH of brain tissue and carbonyl of cardiac tissue, a Kruskal–Wallis test, followed by a post-hoc Dunn test, was used, and values were expressed as median and total range. * $p < 0.05$ vs. C; # $p < 0.05$ vs. CPA. (GSH: reduced glutathione; ASA: ascorbic acid; TBARS: thiobarbituric acid reactive substances; Carbonyl: carbonylated proteins); (n = 8).

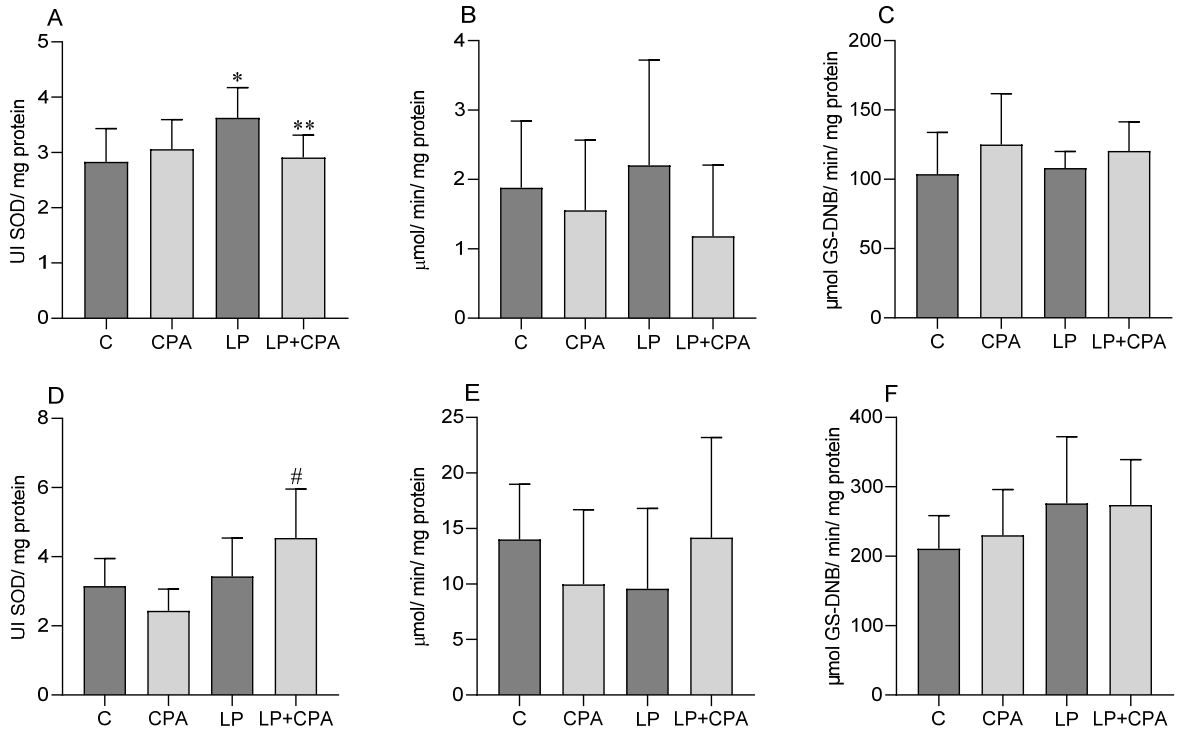

Figure 2. Analysis of enzymatic activities of male Swiss mice treated with LP (protium liposome) and CPA (cyclophosphamide). SOD: superoxide dismutase (**A**), CAT: catalase (**B**), GST: glutathione S-transferase (**C**), e GP_X: glutathione peroxidase (**D**) in the brain; SOD: superoxide dismutase (**E**), CAT: catalase (**F**), GST: glutathione S-transferase. The results are presented as mean ± standard deviation. ANOVA (two-way), followed by Tukey's post-hoc test. For analysis of GST (**B**) in brain tissue and CAT (**E**) in cardiac tissue, the Kruskal–Wallis test, followed by the post-hoc Dunn test, was used, and values were expressed as median and total range. * $p < 0.05$ vs. C; ** $p < 0.05$ vs. LP; # $p < 0.05$ vs. CPA; (n = 8).

Regarding the markers GSH, TBARS, and carbonyl, in the cardiac tissue, no statistically significant changes were identified between the groups investigated. However, regarding the dosage of ASA, a decrease in its levels was observed in the group treated with CPA compared to those in the Control group (Table 6).

4. Discussion

It is known that polyphenols, especially flavonoids, exhibit several pharmacological attributes, such as antioxidant, anti-inflammatory, and anticancer properties, but their usage is still minor compared to their immense therapeutic potential [38]. Although many investigations have been carried out by several authors to study the protective effects of polyphenols, especially flavonoids, obtained from different plant extracts against various types of chemical residues in animal experiments, it is suggested that flavonoids are directly effective for antioxidant activities via the neutralization of free radicals [39]. Many extracts exhibit low bioavailability and rapid degradation, which limits their clinical efficacy. Encapsulating them in liposomes can significantly improve their stability, bioavailability, and specific targeting to tissues [38]. This synergy between phytotherapy and nanosystems allows for the creation of more effective treatments, with less toxicity and fewer side effects, marking a promising advancement in the area [38,40]. In this work, we sought to improve the use of the ethyl acetate fraction from the crude extract of *P. heptaphyllum* in the form of liposomes, and the results obtained revealed several interesting aspects related to the impact of liposomes containing the plant extract during 14 days of preventive treatment against mutagenesis subsequently induced for 24 h with CPA.

The results showed no statistically significant difference in initial or final body weight, as well as feed and water consumption, between the groups analyzed here, and similar data were found in the study by Patias et al. [18], where Wistar rats were treated with the same plant formulation, but at a dose of 1 mg/mL. In a study using mice over a period of 21 days, treatment with CPA (100 mg/kg) and metformin (3 mg/mL) significantly decreased body weight, in addition to reducing the survival rate of the animals [41]. On the other hand, it was observed that the flavonoid quercetin has a significant protective capacity against changes induced by CPA (150 mg/kg) in rats, reversing the decrease in body weight and food intake [42], which suggests that in our study, LP, administered for 14 days, and CPA for 24 h, were not toxic to the point of altering these parameters.

Among our findings regarding organ weight, an important observation was the statistical increase in liver and heart weight in the CPA group compared to the levels in the control group (C). This increase in weight could suggest that CPA may have a direct impact on the morphology and function of these organs. When we refer to liver weight, this may be linked to a possible hepatotoxic effect of CPA, since the liver is often the target of toxicity caused by chemotherapeutic agents or may even be indicative of inflammation or liver damage, often associated with CPA treatment [43]. Another observation was that pretreatment with LP in animals exposed to CPA (LP + CPA) caused a reduction in liver and heart weights compared to those observed in the groups that received only CPA. This finding could indicate that LP may have the ability to reduce the adverse effects caused by CPA in these organs, even if they were only exposed to the mutagenic agent for 24 h. Similarly, Dolgava et al. demonstrated that CPA treatment can affect heart and liver weight in mice [44].

Plasma analysis showed no change in AST in the treated groups, but indicated a decrease in ALP activity, a hepatobiliary marker, in the LP + CPA group, and in creatinine levels, a marker of renal function, for all groups treated with LP and CPA. In contrast, plasma triglyceride levels were elevated in the group treated with CPA alone, possibly due to changes in lipid metabolism caused by CPA. Studies by our group using *P. heptaphyllum* liposomes in the treatment of obesity caused by a high-calorie diet in Wistar rats showed a reduction in AST activity and creatinine levels in both the groups with liposome-containing extract and in the obese group. A similar reduction in ALP was also noted in the obese group and in the group treated with liposomes [18]. On the other hand, in the study by Ref. [14], the administration of EAF from this same plant at a dose of 100 mg/kg in mice for 7 days did not modify the activities of AST and ALP, triglycerides, body weight, and anthropometric parameters. Quercetin was effective in reversing the decrease in weight and the imbalances in hepatic transaminases, urea, and creatinine in rats subjected to oxidative stress by cyclophosphamide (150 mg/kg) [40], which leads us to suggest that the response pattern of LP and CPA significantly depends on the experimental model used, the form of extract administration, the dose, and the treatment time.

Cyclophosphamide is oxidized by P450 enzymes in the liver to become pharmacologically active, where it is converted to highly toxic metabolites—acrolein and phosphoramide mustard [45]—which induce oxidative stress and mutagenesis.

Regarding the micronucleus test, CPA induced a significant increase in PCEMN compared to that in the control, as observed in other studies employing mouse bone marrow cells [46–48]. CPA causes chromosomal damage by covalently binding to DNA and interfering with the cell cycle [49]. The liposome containing the *P. heptaphyllum* extract showed antimutagenic activity in the LP + CPA group. Furthermore, LP per se was not mutagenic. The chemoprotective effect attributed to medicinal plants is largely due to the bioactive compounds present, such as flavonoids [50].

Recent studies report similar results with extracts of plants rich in flavonoids. For example, nanoparticles from *Rhaphidophora pinnata* (50, 100, and 200 mg/kg) demonstrated antimutagenic activity against cyclophosphamide (50 mg/kg) [51]. The methanolic extract of *Dalbergia latifolia* revealed antimutagenic potential against cyclophosphamide (100 mg/kg) [52], and the flavonoid from *Kigelia africana* demonstrated antimutagenic

activity against oxidative stress induced by cyclophosphamide (100 mg/kg) [53]. Considering the anti-inflammatory activity of other compounds present in *P. heptaphyllum*, the essential oil present in the resin of this plant showed, through studies of its chemical composition, cytotoxic action in breast cancer cells (MCF-7), antimicrobial activity, and antimutagenicity in vivo [54]. The possible chemopreventive activity of *P. heptaphyllum* resin essential oil is attributed to monoterpenes, in addition to the absence of cytotoxic and pro-apoptotic effects. Thus, the antioxidant activity of *P. heptaphyllum* [55,56] could potentially explain the antimutagenic activity observed in the present study, since the generation of reactive oxygen species (ROS) and oxidative stress plays a critical role in DNA and chromosome damage [57–59].

Animal research conducted in recent decades has demonstrated that CPA-induced hepatotoxicity is related to oxidative stress [39,43], as it triggers many liver deficiencies due to the generation of ROS [60,61]. The administration of CPA into liver tissue resulted in increased protein carbonylation in the CPA group, which is consistent with the metabolite acrolein that is incorporated into the proteins, generating carbonyl derivatives [62]. On the other hand, there was a decrease in CAT activity in the LP + CPA group compared to that in group C, an increase in GST in the LP + CPA group compared to that in the LP group, and a decrease in GSH in all treated groups (CPA, LP, and LP + CPA), and although not significant, there was a trend towards an increase in GPx for all treatments. In this sense, the GSH depletion caused by CPA is due to the production of the metabolite acrolein, which is able to form conjugations with GSH, reducing its cellular level [63]; however, GSH plays an important role in protecting cells against oxidative damage [64]. Furthermore, studies carried out by Kaushik and Kaur [65] showed the modulation of GST and GPx enzymes in relation to their coenzyme GSH during cold-induced oxidative stress for 21 days and found that enzyme activity increased, even in the presence of low GSH. It is also noteworthy that CPA demonstrated a tendency to increase CAT activity in this organ, and LP seemed to normalize this action, possibly due to the presence of bioactive compounds in the liposomes. Research using EAF from *P. heptaphyllum* in mice also showed the normalization of enzyme activity and GSH levels after paracetamol-induced oxidative stress [14]. Furthermore, a study using triterpenes from *P. heptaphyllum* restored hepatic GSH levels depleted by paracetamol in mice [5].

It is known that GST is involved in cellular detoxification, and its increase in the LP + CPA group may be related to an adaptive response of the liver to the stress caused by CPA. A study with *Carica papaya* Linn extract triggered an increase in GST and CAT activity in the livers of mice exposed to a single dose of CPA (75 mg/kg) [46]. Although there were changes in the redox state of the liver, no changes were observed in the immunological and histological parameters in the tissues of the groups analyzed. Similar to the results observed in this study, Patias et al. [18] did not see changes per se in LP for histological analyses and for TNF-α, IL-6, IL-17, but observed a positive effect for IL-10, and in obese animals, for IL-1β, which suggests that the animal species interferes with the cytokine response.

In renal tissue, markers of lipid and protein damage were not modified by the treatments, except in the case of LP, which showed a physiological reduction in TBARS. Furthermore, it revealed a significant increase in GPx activity in the LP + CPA group and a reduction in GST for all treatments. Contrary to our findings, studies with *Solanum scabrum* and *Cola verticillata* extracts, plants rich in flavonoids, demonstrated a decrease in renal GPx activity in rats exposed to CPA (100 mg/kg) [66]. It appears that the *P. heptaphyllum* liposome induced a response that requires greater GPx activity, possibly signaled by an additional production of hydrogen peroxide and also due to the reduction in GST. In the work of Patias et al. [14], EAF did not promote changes in GST activity per se, such as those noted in this study. On the other hand, in this study, the form of delivery of the extract in the liposomes in the group treated only with extract resulted in a decrease in TBARS. Although we observed changes in some biomarkers, the mutagenesis inducer was not effective in causing damage within 24 h of administration in the renal tissue, and consequently, the

LP did not cause the activation of the immune response or the immunomodulation of the cytokines.

In brain tissue, we observed an increase per se in SOD enzyme activity in the LP group, and its activity returned to control levels in the presence of CPA compared to the levels in the LP group, which suggests that the presence of CPA may interfere with the activity of this enzyme in this tissue. The brain is a tissue composed mostly of lipids; thus, it is highly vulnerable to oxidation, and it is noteworthy that CPA did not induce changes to the point of causing damage in this tissue, which can be observed by the absence of statistical differences between the tissues of the groups studied. There are reports that curcumin improves redox balance and shows protection against oxidative damage induced by cyclophosphamide (150 mg/kg) in the brains of rats [67], and quercetin showed promising neuroprotective effects against brain oxidative damage induced by cyclophosphamide in several studies [42,68]. Furthermore, flavonoids such as quercetin, apigenin, and genistein have demonstrated the ability to reverse dysfunction caused by oxidative stress in brain endothelial cells [69]. These findings collectively highlight the neuroprotective potential of flavonoids in combating CPA-induced brain stress. In addition, we can suggest that the short time (24 h) of CPA treatment in this study was insufficient to generate brain damage, but the 14-day treatment with LP was sufficient to positively modulate SOD enzyme activity.

In cardiac tissue, there was a tendency for reduced SOD activity in the CPA group, but pretreatment with LP promoted a significant increase in this activity, suggesting that the liposome promoted an improvement in enzymatic activity. On the other hand, there was a statistical decrease in ASA levels in the CPA group, indicating a general reduction in the antioxidant capacity of this marker in cardiac tissue due to treatment with CPA, and the *P. heptaphyllum* liposome was unable to modify this parameter. Unlike the results of our investigation, the work of Ye et al. [70], in which animals were orally treated with the flavonoid chrysin (25 and 50 mg/kg/day) for 35 days and exposed to cyclophosphamide (100 mg/kg) once a week for four weeks, showed that the activities of cardiac antioxidant enzymes, such as SOD and CAT, as well as the GSH levels, were suppressed. Studies by Luiz et al. [45] also did not observe major changes in oxidative stress parameters in the hearts of mice treated with CPA (75 mg/kg) for 24 h, as was the case in our study. It is likely that this short time of exposure to CPA was not enough to cause many changes in the parameters investigated in this study for the brain and heart, but considering the results for the liver, which is the organ that metabolizes this drug, and the kidney, which is the site of excretion, these tissues did demonstrate that they are more affected by the treatment, as observed in our findings.

5. Final Considerations

The data show that *P. heptaphyllum* liposomes may have an antimutagenic effect, suggesting that they may act as a protective agent against DNA damage caused by CPA, including providing protection against oxidative stress, as evidenced by increased activities of the antioxidant enzymes SOD in the brain and heart, and by the increase in GPx in the kidneys. However, the depletion of GSH in the liver and brain suggests the use of antioxidants for the metabolism of molecules generated in these tissues. The improvements in ALP and creatinine markers indicate a possible hepatoprotective and renoprotective effect. Overall, these results highlight the need for further studies to fully elucidate the mechanisms of action of *P. heptaphyllum* liposomes.

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References

1. Kanno, T.Y.N.; Sensiate, L.A.; de Paula, N.A.; Salles, M.J.S. Toxic effects of different doses of cyclophosphamide on the reproductive parameters of male mice. *Braz. J. Pharm. Sci.* **2009**, *45*, 313–319. [CrossRef]
2. Alrefaei, A.E.; Alzahrani, M.A.; Alsuhaim, S.A. Cyclophosphamide related toxicity; a systematic review. *Int. J. Med. Dev. Ctries.* **2022**, *6*, 740–747. [CrossRef]
3. Mirkes, P.E. Cyclophosphamide teratogenesis: A review. *Teratog. Carcinog. Mutagen.* **1985**, *5*, 75–88. [CrossRef] [PubMed]
4. Salem, M.; Nassef, M.; Abdel-Salam, S.; Alshorbagy, I.; Ata, A. Cytotoxic and anti-tumor effects of the chemotherapeutic drug cyclophosphamide in tumor bearing mice is time and dose dependent. *Egypt. J. Zool.* **2014**, *62*, 131–146. [CrossRef]
5. Oliveira, F.A.; Chaves, M.H.; Almeida, F.R.; Lima, R.C.; Silva, R.M.; Maia, J.L.; Brito, G.A.A.; Santos, F.A.; Rao, V.S. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol.* **2005**, *98*, 103–108. [CrossRef]
6. Shen, Y.; Hao, X. Natural product sciences: An integrative approach to the innovations of plant natural products. *Sci. China Life Sci.* **2020**, *63*, 1634–1650. [CrossRef]
7. Chopra, B.; Dhingra, A.K. Natural products: A lead for drug discovery and development. *Phytother. Res.* **2021**, *35*, 4660–4702. [CrossRef]
8. Islam, R.; Kabir, M.F.; Alam, R.; Dahr, R.; Rana, M.N.; Islam, E.; Parvin, S.; Hossain, A. Sedative, membrane stability, cytotoxic and antioxidant properties of methanol extract of leaves of *Protium serratum* Wall. *Asian Pac. J. Trop. Dis.* **2014**, *4*, 928–933. [CrossRef]
9. Nogueira, A.O.; Oliveira, Y.I.S.; Adjafre, B.L.; de Moraes, M.E.A.; Aragão, G.F. Pharmacological effects of the isomeric mixture of alpha and beta amyrin from *Protium heptaphyllum*: A literature review. *Fundam. Clin. Pharm.* **2019**, *33*, 4–12. [CrossRef] [PubMed]
10. Tafurt-García, G.; Muñoz-Acevedo, A. Volatile metabolites present in *Protium heptaphyllum* (Aubl.) March. collected in Tame (Arauca—Colombia). *Lat. Am. Caribb. Bull. Med. Aromat. Plants* **2012**, *11*, 223–232. Available online: https://pesquisa.bvsalud.org/portal/resource/pt/lil-647661 (accessed on 2 June 2024).
11. Bandeira, P.N.; Pessoa, O.D.L.; Trevisan, M.T.S.; Lemos, T.L.G. Secondary metabolites of *Protium heptaphyllum* March. *Quim. Nova* **2002**, *25*, 1078–1080. [CrossRef]
12. Marques, D.D.; Sartori, R.A.; Lemos, T.L.G.; Machado, L.L.; Souza, J.S.N.; Monte, F.J.Q. Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*. *Acta Amaz.* **2010**, *40*, 227–230. [CrossRef]
13. Maia, R.M.; Barbosa, P.R.; Cruz, F.G.; Roque, N.F.; Fascio, M. Triterpenes from the resin of *Protium heptaphyllum* March (Burseraceae): Characterization in binary mixtures. *Quím. Nova* **2000**, *23*, 623–626. [CrossRef]
14. Patias, N.S.; Sinhorin, V.D.G.; de Moura, F.R.; da Cunha, A.P.F.; Lima, R.R.S.; Costa, R.J.; da Costa, T.B.; Cavalheiro, L.; Bicudo, R.C.; Sinhorin, A.P. Identification of flavonoids by lc-ms/ms in leaves extract from *Protium heptaphyllum* (Aubl.) March and antioxidant activity in mice. *Nat. Prod. J.* **2021**, *11*, 715–727. [CrossRef]
15. Devi, S.; Kumar, V.; Singh, S.K.; Dubey, A.K.; Kim, J.J. Flavonoids: Potential Candidates for the Treatment of Neurodegenerative Disorders. *Biomedicines* **2021**, *9*, 99. [CrossRef] [PubMed]
16. Durairaj, V.; Hoda, M.; Shakya, G.; Babu, S.P.; Rajagopalan, R. Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pac. J. Trop. Med.* **2014**, *7S1*, S398–S404. [CrossRef]
17. Bishnu, K.; Jyoti, S. A Review Study of Importance of Herbal Medicine. *Int. J. Multidiscip. Res.* **2023**, *5*. [CrossRef]
18. Patias, N.S.; Queiroz, E.A.I.F.; Ferrarini, S.R.; Bomfim, G.F.; Aguiar, D.H.; Sinhorin, A.P.; Bello, A.A.; Silva, G.V.F.; Cavalheiro, L.; Sinhorin, V.D.G. Effect of liposomal extract of *Protium heptaphyllum* (Alb.) March in the treatment of obesity induced by a high-calorie diet. *Biology* **2024**, *13*, 535. [CrossRef]

19. Anand, A.; Gautam, P.; Ojha, S. Application of Nanotechnology for Herbal Medicine Development: A Review. *Lett. Drug Des. Discov.* **2023**, *21*, 1325–1333. [CrossRef]
20. Gad, S.; ElGogary, R.; Geneidi, A.; Hathout, R. Lipid nanocarriers encapsulating herbal drugs for brain diseases therapy. *Arch. Pharm. Sci. Ain Shams Univ.* **2023**, *7*, 60–86. [CrossRef]
21. Halder, M.; Jha, S. Medicinal Plants and Bioactive Phytochemical Diversity: A Fountainhead of Potential Drugs against Human Diseases. In *Medicinal Plants: Biodiversity, Biotechnology Conservation*; Jha, S., Halder, M., Eds.; Springer: Singapore, 2023; Volume 33. [CrossRef]
22. Hua, S.; WU, S.Y. The use of lipid-based nanocarriers for targeted pain therapies. *Front. Pharmacol.* **2013**, *4*, 143. [CrossRef] [PubMed]
23. Malone, M.H. The pharmacological evaluation of natural products—General and specific approaches to screening ethnopharmaceuticals. *J. Ethnopharmacol.* **1983**, *8*, 127–147. [CrossRef]
24. Bhat, N.; Kalthur, S.G.; Padmashali, S.; Monappa, V. Toxic Effects of Different Doses of Cyclophosphamide on Liver and Kidney Tissue in Swiss Albino Mice: A Histopathological Study. *Ethiop. J. Health Sci.* **2018**, *28*, 711–716. [CrossRef] [PubMed]
25. MacGregor, J.T.; Heddle, J.A.; Hite, M.; Margolin, B.H.; Ramel, C.; Salamone, M.F.; Tia, R.R.; Wild, D. Guidelines for the conduct of micronucleus assay in mammalian bone marrow erythrocytes. *Mutat. Res.* **1987**, *189*, 103–112. [CrossRef] [PubMed]
26. Nelson, D.P.; Kiesow, L.A. Enthalpy of decomposition of hydrogen peroxide by catalase at 25 degrees C (with molar extinction coefficients of H_2O_2 solutions in the UV). *Anal. Biochem.* **1972**, *49*, 474–478. [CrossRef]
27. Misra, H.P.; Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **1972**, *247*, 3170–3175. Available online: https://pubmed.ncbi.nlm.nih.gov/4623845/ (accessed on 2 July 2024). [CrossRef]
28. Habig, W.H.; Pabst, M.J.; Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139. Available online: https://pubmed.ncbi.nlm.nih.gov/4436300/ (accessed on 16 July 2024). [CrossRef] [PubMed]
29. Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158–169. Available online: https://pubmed.ncbi.nlm.nih.gov/6066618/ (accessed on 4 July 2024).
30. Sedlack, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* **1968**, *25*, 192–205. [CrossRef]
31. Roe, J.H. Chemical determination of ascorbic, dehydroascorbic, and diketogulonic acids. *Methods Biochem. Anal.* **1954**, *1*, 115–139. [CrossRef]
32. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310. [CrossRef] [PubMed]
33. Colombo, G.; Clerici, M.; Garavaglia, M.E.; Giustarini, D.; Rossi, R.; Milzani, A.; Dalle-Donne, I. A step-by-step protocol for assaying protein carbonylation in biological samples. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2016**, *1019*, 178–190. [CrossRef] [PubMed]
34. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
35. Barros, C.S.L.; Rissi, D.R. Down the rabbit hole: A quick guide for histopathology description. *Pesq. Vet. Bras.* **2021**, *41*, e06927. [CrossRef]
36. Pereira, C.A.B.; Rabello, G.M.N. Statistical test to compare proportions in cytogenetic problems. Mutagenesis, carcinogenesis and teratogenesis: Methods and evaluation criteria. Ribeirão Preto. *Braz. Soc. Genet.* **1991**, 113–121.
37. Waters, M.D.; Brady, A.L.; Stack, H.F.; Brockman, H.E. Antimutagenicity profiles for some model compounds. *Mutat. Res.* **1990**, *1*, 57–85. [CrossRef]
38. Ranjbar, S.; Emamjomeh, A.; Sharifi, F.; Zarepour, A.; Aghaabbasi, K.; Dehshahri, A.; Sepahvand, A.M.; Zarrabi, A.; Beyzaei, H.; Zahedi, M.M.; et al. Lipid-Based Delivery Systems for Flavonoids and Flavonolignans: Liposomes, Nanoemulsions, and Solid Lipid Nanoparticles. *Pharmaceutics* **2023**, *15*, 1944. [CrossRef]
39. Al-Kubaisy, K.N.; Al-Essa, L.Y.; Shawagfeh, M.T. Stimulation of Hepatocytes Repair by Fruit Juice of Opuntia ficus indica in Anti Cancer Drug Cyclophosphamide (CP)-Induced Liver Toxicity in Mice. *Annu. Res. Rev. Biol.* **2016**, *10*, 1–8. [CrossRef]
40. Roszkowski, S. Application of Polyphenols and Flavonoids in Oncological Therapy. *Molecules* **2023**, *28*, 4080. [CrossRef]
41. Alhowail, A.; Sajid, S.; Almogbel, Y.; Rabbani, S.I.; Alsharidah, M. Effect of Cyclophosphamide and Its Combination with Metformin on the Survival Rate in Mice. *J. Pharm. Res. Int.* **2019**, *30*, 1–6. [CrossRef]
42. Onaolapo, A.Y.; Ojo, F.O.; Onaolapo, O.J. Biflavonoid quercetin protects against cyclophosphamide-induced organ toxicities via modulation of inflammatory cytokines, brain neurotransmitters, and astrocyte immunoreactivity. *Food Chem. Toxicol.* **2023**, *178*, 113879. [CrossRef] [PubMed]
43. Zahraa, Z.A.; Haider, H.H.; Amer, A. Toxic effects of Cyclophosphamide on Hepatic and Kidney tissues in Albino Mice Model. *Res. J. Pharm. Technol.* **2022**, *15*, 4655–4659. [CrossRef]
44. Ying, Y.; Hu, J.; Li, L.; Li, Y.; Wang, S.; Qu, H. The Influence of Cyclophosphamide on Growth Peripheral Blood Biochemidtry Index and Organ Coefficient of SD Rat. *Chin. J. Cancer* **2010**, *37*, 18–21. Available online: https://www.chvm.net/EN/Y2010/V37/I10/18 (accessed on 2 June 2024).
45. Golan, E.D.; Tashjian, H.A.; Armstrong, J.E.; Armstrong, W.A. *Princípios de Farmacologia: A Base Fisiopatológica da Farmacoterapia*, 3rd ed.; Guanabara Koogan: Rio de Janeiro, Brazil, 2014.

46. Luiz, T.C.; Cunha, A.P.S.; Aguiar, D.; Sugui, M.M.; Bicudo, R.C.; Sinhorin, A.P.; Sinhorin, V.D.G. Antioxidant potential of *Carica papaya Linn* (Caricaceae) leaf extract in mice with cyclophosphamide induced oxidative stress. *Sci. Med.* **2020**, *30*, e34702. [CrossRef]
47. Bragante, W.; Sinhorin, V.D.G.; Sugui, M.M.; Cunha, A.P.S.; Santos, W.B.; Sinhorin, A.P. In vivo mutagenic effects and oxidative stress parameters evaluation of cypermethrin and benzoate of emamectin and their mixtures in female mice. *J. Environ. Sci. Health Part B* **2022**, *57*, 211–219. [CrossRef] [PubMed]
48. Sinhorin, V.D.G.; Cordeiro Luiz, T.; Baldissera, L.; Simões, C.A.P.; Bressan, S.W.; Lopes, B.A.J.; Sugui, M.M.; Valadão, D.M.S. Prophylactic action of the isotonic drink made from the skins of the fruit of *Oenocarpus bacaba Mart*. (Arecaceae) against cyclophosphamide-induced oxidative stress in mice. *Native* **2024**, *11*, 251–258. [CrossRef]
49. Ribeiro, L.R.; Salvadori, D.M.; Marques, E.K. *Mutagênse Ambiental*, 1st ed.; Editora Ulbra: Canoas, RS, Brazil, 2024; Available online: https://www.researchgate.net/publication/284581917_Mutagenese_Ambiental (accessed on 11 June 2024).
50. Gomes-Carneiro, M.R.; Viana, M.E.; Felzenszwalb, I.; Paumgartten, F.J. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (−)-alpha-pinene in the Salmonella/microsome assay. *Food Chem. Toxicol.* **2005**, *43*, 247–252. [CrossRef]
51. Masfria, M.; Marianne, M.; Permata, Y.M.; Octavio, S.; Mulyani, S. Antimutagenic activity of nanoparticles of *Rhaphidophora pinnata* leaves in mice using micronucleus assay. *J. Adv. Pharm. Technol. Res.* **2021**, *12*, 232–235. [CrossRef] [PubMed]
52. Yadav, S.; Kumar, S. Evaluation of Mutagenic Effect (Antimutagenic) of *Dalbergia Latifolia* on Swiss Albino Mice. *Int. J. Toxicol. Pharmacol. Res.* **2015**, *7*, 80–84. Available online: https://www.researchgate.net/publication/379025686 (accessed on 20 July 2024).
53. Vasantha, K.C.; Nagarathna, P.K.M.; Sudhir, C.K.; Sri Sainadh, N. Evaluation of Antimutagenic Effect of Flavonoid of *Kigelia Africana* on Swiss-Albino Mice. *Int. J. Pharm. Sci. Rev. Res.* **2013**, *21*, 105–108. Available online: https://www.researchgate.net/publication/287919880_Evaluation_of_antimutagenic_effect_of_flavonoid_of_Kigelia_Africana_on_Swiss-Albino_mice (accessed on 22 July 2024).
54. Lima, E.M.; Cazelli, D.S.; Pinto, F.E.; Mazuco, R.A.; Kalil, I.C.; Lenz, D.; Scherer, R.; de Andrade, T.U.; Endringer, D.C. Essential Oil from the Resin of *Protium heptaphyllum*: Chemical Composition, Cytotoxicity, Antimicrobial Activity, and Antimutagenicity. *Pharmacogn. Mag.* **2016**, *12*, S42–S46. [CrossRef] [PubMed]
55. Araujo, D.A.; Takayama, C.; Faria-de, F.M.; Socca, E.A.; Dunder, R.J.; Manzo, L.P.; Luiz-Ferreira, A.; Souza-Brito, A.R.M. Gastroprotective effects of essential oil from *Protium heptaphyllum* on experimental gastric ulcer models in rats. *Braz. J. Pharmacogn.* **2011**, *21*, 721–729. [CrossRef]
56. Bandeira, P.N.; Fonseca, A.M.; Costa, S.M.; Lins, M.U.; Pessoa, O.D.; Monte, F.J.; Nogueira, N.; Lemos, T.L.G. Anti-bacterial and anti-oxidant activities of the essential oil of resin of *Protium heptaphyllum*. *Nat. Prod. Commun.* **2006**, *1*, 117–120. [CrossRef]
57. Soto-Reyes, E.; Del Razo, L.M.; Valverde, M.; Rojas, E. Role of the alkali labile sites, reactive oxygen species and antioxidants in DNA damage induced by methylated trivalent metabolites of inorganic arsenic. *Biometals* **2005**, *18*, 493–506. [CrossRef]
58. Ahmed, M.K.; Habibullah-Al-Mamun, M.; Hossain, M.A.; Arif, M.; Parvin, E.; Akter, M.S.; Khan, M.S.; Islam, M.M. Assessing the genotoxic potentials of arsenic in tilapia (*Oreochromis mossambicus*) using alkaline comet assay and micronucleus test. *Chemosphere* **2011**, *84*, 143–149. [CrossRef]
59. Shao, B.; Zhu, L.; Dong, M.; Wang, J.; Wang, J.; Xie, H.; Zhang, Q.; Du, Z.; Zhu, S. DNA damage and oxidative stress induced by endosulfan exposure in zebrafish (*Danio rerio*). *Ecotoxicology* **2012**, *21*, 1533–1540. [CrossRef] [PubMed]
60. Abbas, K.; Batool, U.; Abdul, S.M.; Sana, S. Estresse oxidativo: Uma espada de dois gumes. *BioSight* **2024**, *2*, 4–12. [CrossRef]
61. Sadasivam, N.; Kim, Y.J.; Radhakrishnan, K.; Kim, D.K. Estresse oxidativo, integridade genômica e doenças hepáticas. *Moléculas* **2022**, *27*, 3159. [CrossRef]
62. Moghe, A.; Ghare, S.; Lamoreau, B.; Mohammad, M.; Barve, S.; McClain, C.; Joshi-Barve, S. Molecular mechanisms of acrolein toxicity: Relevance to human disease. *Toxicol. Sci.* **2015**, *143*, 242–255. [CrossRef]
63. Nafees, S.; Rashid, S.; Ali, N.; Hasan, S.K.; Sultana, S. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: Role of NFjB/MAPK pathway. *Chem. Biol. Interact.* **2015**, *231*, 98–107. [CrossRef]
64. Golubkova, A.; Leiva, T.; Snyder, K.; Schlegel, C.; Bonvicino, S.M.; Agbaga, M.-P.; Brush, R.S.; Hansen, J.M.; Vitiello, P.F.; Hunter, C.J. Response of the Glutathione (GSH) Antioxidant Defense System to Oxidative Injury in Necrotizing Enterocolitis. *Antioxidants* **2023**, *12*, 1385. [CrossRef] [PubMed]
65. Kaushik, S.; Kaur, J. Chronic cold exposure affects the antioxidant defense system in various rat tissues. *Clin. Chim. Acta* **2003**, *333*, 69–77. [CrossRef] [PubMed]
66. Angie, M.B.; Paka, D.; Ntentie, G.; Ngondi, D.; Enyong, O. Protective effect of hydroethanolic extracts of *Solanum scabrum* and *Cola verticillata* against cyclophosphamide induced toxicity in female rats. *J. Food Res.* **2014**, *3*, 18–30. [CrossRef]
67. Akomolafe, S.F.; Olasehinde, T.A.; Oyeleye, S.I.; Aluko, T.B.; Adewale, O.O.; Ijomone, O.M. Curcumin Administration Mitigates Cyclophosphamide-Induced Oxidative Damage and Restores Alteration of Enzymes Associated with Cognitive Function in Rats' Brain. *Neurotox. Res.* **2020**, *38*, 199–210. [CrossRef] [PubMed]
68. AbdElrazek, D.A.; Ibrahim, M.A.; Hassan, N.H.; Hassanen, E.I.; Farroh, K.Y.; Abass, H.I. Neuroprotective effect of quercetin and nano-quercetin against cyclophosphamide-induced oxidative stress in the rat brain: Role of Nrf2/ HO-1/Keap-1 signaling pathway. *Neurotoxicology* **2023**, *98*, 16–28. [CrossRef]

69. Tan, Q.; Yan, X.; Chen, L.; Jiang, K.; Guo, Z. Dietary flavonoids protect human brain microvascular endothelial cell from oxidative stress-induced dysfunction. *Mol. Cell. Toxicol.* **2024**, 1–11. [CrossRef]
70. Ye, B.; Ling, W.; Wang, Y.; Jaisi, A.; Olatunji, O.J. Protective Effects of Chrysin against Cyclophosphamide-Induced Cardiotoxicity in Rats: A Biochemical and Histopathological Approach. *Chem. Biodivers.* **2022**, *19*, e202100886. [CrossRef]

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REFERÊNCIAS GERAIS

Anand A., Gautam P., Ojha S. Application of Nanotechnology for Herbal Medicine Development: A Review, Letters in Drug Design & Discovery. 2023, 20. https://doi.org/10.2174/1570180820666230308105723

Aragão, GF; Carneiro, LM; Junior, AP; Vieira, LC; Bandeira, PN; Lemos, TL; Viana, GS. A possible mechanism for anxiolytic and antidepressant effects of alpha- and beta-amyrin from *Protium heptaphyllum* (Aubl.) March. *Farmacol. Bioqu. Comport*. **2006**, 85(4), 827–834. https://doi.org/10.1016/j.pbb.2006.11.019

Bandeira, P. N., Pessoa, O. D. L., Trevisan, M. T. S., & Lemos, T. L. G. Metabólitos secundários de *Protium heptaphyllum* march. *Quím. Nova*. **2002**, 25(6b), 1078–1080. https://doi.org/10.1590/S0100-40422002000700006

Cabral, R. S. C., Alves, C. C. F., Batista, H. R. F., Sousa, W. C., Abrahão, I. S., Crotti, A. E. M., Santiago, M. B., Martins, C. H. G., & Miranda, M. L. D. Chemical composition of essential oils from different parts of Protium heptaphyllum (Aubl.) Marchand and their in vitro antibacterial activity. *Prod. Nacional. Res*. **2020**, 34(16), 2378–2383. https://doi.org/10.1080/14786419.2018.1536659

Carvalho, KM; de Melo, TS; de Melo, KM; Quinderé, AL; de Oliveira, FT; Viana, AF; Nunes, PI; Quetz, JD; Viana, DA; da Silva, AA; Havt, A; Fonseca, SG; Chaves, MH; Rao, VS; Santos, FA. Amyrins from Protium heptaphyllum Reduce High-Fat Diet-Induced Obesity in Mice via Modulation of Enzymatic, Hormonal and Inflammatory Responses. *Planta med*. **2017**, 83(3-04), 285–291. https://doi.org/10.1055/s-0042-114222

Carvalho, KM; Marinho Filho, JD; de Melo, TS; Araújo, AJ; Quetz, JdaS; da Cunha, M doP; de Melo, KM; da Silva, AA; Tomé, AR; Havt, A; Fonseca, SG; Brito, GA; Chaves, M H; Rao, VS; Santos, FA. The Resin from Protium heptaphyllum Prevents High-Fat Di-et-Induced Obesity in Mice: Scientific Evidence and Potential Mechanisms. *Evid. Based Complement. Alternat. Med.* **2015**, 2015, 106157. https://doi.org/10.1155/2015/106157

Chauhan, D., Yadav, P. K., Sultana, N., Agarwal, A., Verma, S., Chourasia, M. K., & Gayen, J. R. Advancements in nanotechnology for the delivery of phytochemicals. *J. Integr. Med*. **2024**, 22(4), 385–398. https://doi.org/10.1016/j.joim.2024.04.005

David, Bender. Radicais livres e nutrientes antioxidantes. In: RODWELL, V. W. *Bioquímica Ilustrada de Harper*. Artemed. Porto Alegre: Grupo A, **2021**. 9786558040033. Disponível em: https://integrada.minhabiblioteca.com.br/#/books/9786558040033/. Acesso em: 21 set. 2024.

Durairaj V., Hoda M., Shakya G., Badu S.P.P., Rajacopalan R. Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass. *Asian Pac. J. Trop. Med*. **2014**, 7,398–404. https://doi.org/10.1016/S1995-7645(14)60265-0

de Lima, E. M., Cazelli, D. S., Pinto, F. E., Mazuco, R. A., Kalil, I. C., Lenz, D., Scherer, R., de Andrade, T. U., Endringer, D. C. Essential Oil from the Resin of Protium heptaphyllum: Chemical Composition, Cytotoxicity, Antimicrobial Activity, and Antimutagenicity. *Pharmacogn Mag*. **2016**, 12(1): S42-S46. https://doi.org/10.4103/0973-1296.176113

Gad, S., ElGogary, R., Geneidi, A., Hathout, R. Lipid nanocarriers encapsulating herbal drugs for brain diseases therapy. *Arch. Pharm. Sci. ASU*. **2023**, 7(1), pp. 60-86. doi:10.21608/aps.2023.202432.1115

Holanda Pinto, SA; Pinto, LM; Cunha, GM; Chaves, MH; Santos, FA; Rao, VS. Anti-inflammatory effect of alpha, beta-amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in a rat model of acute periodontitis. *Inflammopharmacology*, **2008**, 16(1), 48-52. https://doi.org/10.1007/s10787-007-1609-x

Islam, R., Kabir, M.F., Alam, R., Dahr, R., Rana, M. N., Islam, E., Parvin, S., Hossain, A. Sedative, membrane stability, cytotoxic and antioxidant properties ofmethanol extract of leaves of *Protium serratum* Wall. *Asian Pac. J. Trop. Dis*. **2014**, 4(2), 928 – 933.

Kumar Vishwakarma D, Narayan Mishra J, Kumar Shukla A, et al. (2024) Phytosomes as a Novel Approach to Drug Delivery System. *Pharmaceutical Science*. IntechOpen. 2024, Available at: http://dx.doi.org/10.5772/intechopen.113914.

Maia R. M., Barbosa, P. R. Cruz, F. G. Roque, N. F. Fascio, M. 2000. Triterpenos da resina de *Protium heptaphyllum* March. (Bourseraceae): Caracterização em misturas binárias. *Quím. Nova*. **2000**, 23, 623-626.

Marques, D. D., Sartori, R. A., Lemos, T. L. G., Machado, L. L., Souza De, J. S. N., Monte, F. J. Q. Chemical composition of the essential oils from two subspecies of *Protium heptaphyllum*. *Acta Amaz*. 2010, 40. p. 227 – 230.

Oliveira, FA; Chaves, MH; Almeida, FR; Lima, RC; Jr. Silva, RM; Maia, JL; Brito, GA; Santos, F.A; Rao, VS. Protective effect of alpha- and beta-amyrin, a triterpene mixture from Protium heptaphyllum (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J. Ethnopharmacol*. **2005**, 98(1-2), 103–108. https://doi.org/10.1016/j.jep.2005.01.036

Patias S. N., Gindri S. D. V. e Sinhorin P. A. Potential antioxidants and other biological activities of Protium heptaphyllum (Aubl.). March: Mini-Review. *Nat. Prod. J.* **2023**, 13(7): e090223213549. https://dx.doi.org/10.2174/2210315513666230209143555

Patias, N. S., de Queiroz, E. A. I. F., Ferrarini, S. R., Bomfim, G. F., Aguiar, D. H., Sinhorin, A. P., Bello, A. A., da Silva, G. V. F., Cavalheiro, L., & Sinhorin, V. D. G. Effect of Liposomal *Protium heptaphyllum* (Alb.) March Extract in the Treatment of Obesity Induced by High-Calorie Diet. *Biology*, **2024**, *13*(7), 535. https://doi.org/10.3390/biology13070535

Patias, N. S., de Queiroz, E. A. I. F., Ferrarini, S. R., Bomfim, G. F., Aguiar, D. H., Sinhorin, A. P., Bello, A. A., da Silva, G. V. F., Cavalheiro, L., & Sinhorin, V. D. G. Effect of Liposomal *Protium heptaphyllum* (Alb.) March Extract in the Treatment of Obesity Induced by High-Calorie Diet. *Biology*, **2024**, *13*(7), 535. https://doi.org/10.3390/biology13070535

Patias, N. S., Sinhorin, V. D. G., Ferneda, A. J. L. B., Ferneda, J. M. A., Sugui, M. M., Ferrarini, S. R., Bomfim, G. F., Lopes, J. W., Antoniassi, N. A. B., Cavalheiro, L., Domingues, N. L. C., & Sinhorin, A. P. Study of Liposomes Containing Extract from the Leaves of *Protium heptaphyllum* (Aubl.) March in Animals Submitted to a Mutagenic Model Induced by Cyclophosphamide. *Biology*. 2024, *13*(9), 706. https://doi.org/10.3390/biology13090706

Patias, NS; Sinhorin, VDG; de Moura, F.R; da Cunha, APF; Lima, RRS; Costa, RJ; da Costa, TB; Cavalheiro, L; Bicudo, RC; Sinhorin, AP. Identification of flavonoids by lc-ms/ms in

leaves extract from Protium heptaphyllum (Aubl.) March and antioxidant activity in mice. *Nat. Prod. J.* **2021**, 11(5), 715-727. https://doi.org/10.2174/2210315510999200817165311

Rudiger, A. L., Valdir Junior, F. V. Chemodiversity of Ursane- and Oleanane-Type Triterpenes in Amazonian Burseraceae Oleoresins. *Chem Biodivers*. **2013**, 10. p. 1142 – 1153. https://doi.org/10.1002/cbdv.201200315