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Hepatotoxicity induced by *Peganum harmala* and the protective potential of medicinal plant in rats

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Dedication

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Dedication

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Dedication

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To the soul of my beloved father, may Allah have mercy on him. Although you are no longer by my side, your memory remains forever in my heart. I pray that this work will be a light in your grave and a continuous charity for you.

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Abstract

Peganum harmala is known for its various pharmacological activities, but excessive administration may lead to severe liver damage associated with oxidative stress and cellular alterations. In contrast, *Silybum marianum* is recognized for its antioxidant and hepatoprotective properties. This study investigated the hepatotoxicity of *Peganum harmala* (PGE) exposure on physiological, biochemical, and oxidative stress parameters in Albino-Wistar rats, while also evaluating the hepatoprotective role of *Silybum marianum* (SME).

20 female rats were divided into four experimental groups: control (receive drinking water and standard food), PHE-only (received a decoction prepared from 60 mg/kg of *Peganum harmala*), SME-only (received a dose of 80 mg/kg) and PHE+SME (received *Silybum marianum* 80 mg/kg followed one hour later by *Peganum harmala* 60 mg/kg). The treatments were administered orally for 45 consecutive days.

The results demonstrated that administration of *Peganum harmala* induced significant hepatotoxicity, characterized by elevated liver enzyme levels (ALAT, ASAT), altered metabolic markers (Triglycerides, Albumin), and increased oxidative stress, as evidenced by significant changes in CAT activity and GSH levels. Conversely, treatment with *Silybum marianum* showed a protective effect by improving biochemical parameters and reducing hepatic damage. These findings suggest that *Silybum marianum* may attenuate liver injury caused by *Peganum harmala* through its antioxidant and hepatoprotective activities.

Keywords: *Peganum harmala*, *Silybum marianum*, hepatotoxicity, hepatoprotection, oxidative stress

Resumé

Peganumharmala est connue pour ses diverses activités pharmacologiques, mais une administration excessive peut entraîner une atteinte hépatique sévère associée au stress oxydatif et à des altérations cellulaires. En revanche, *Silybummarianum* est reconnue pour ses propriétés antioxydantes et hépatoprotectrices. Cette étude a évalué l'hépatotoxicité induite par l'exposition à *Peganumharmala* (PHE) sur les paramètres physiologiques, biochimiques et du stress oxydatif chez des rats Albino-Wistar, tout en examinant le rôle hépatoprotecteur de *Silybummarianum* (SME).

Vingt rats femelles ont été répartis en quatre groupes expérimentaux : un groupe témoin (recevant de l'eau de boisson et une alimentation standard), un groupe PHE (recevant une décoction préparée à partir de *Peganumharmala* à la dose de 60 mg/kg), un groupe SME (recevant *Silybummarianum* à la dose de 80 mg/kg) et un groupe PHE+SME (recevant *Silybummarianum* à la dose de 80 mg/kg, suivi une heure plus tard par *Peganumharmala* à la dose de 60 mg/kg). Les traitements ont été administrés par voie orale pendant 45 jours consécutifs.

Les résultats ont démontré que l'administration du *Peganumharmalaa* induit une hépatotoxicité significative, caractérisée par une élévation des enzymes hépatiques (ALAT et ASAT), une altération des marqueurs métaboliques (Triglycérides et Albumin), ainsi qu'un stress oxydatif accru, mis en évidence par des changements significatifs dans l'activité de la CAT et les taux de GSH. En revanche, le traitement par *Silybummarianum* a exercé un effet protecteur en améliorant les paramètres biochimiques et en réduisant les lésions hépatiques. Ces résultats suggèrent que *Silybummarianum* pourrait atténuer les lésions hépatiques induites par *Peganumharmala* grâce à ses activités antioxydantes et hépatoprotectrices.

Mots-clés: *Peganumharmala*, *Silybummarianum*, hépatotoxicité, hépatoprotection, stress oxydatif.

ملخص

يُعرف الحرمل بتعدد خصائصه الدوائية، إلا أن الإفراط في استعماله قد يؤدي إلى حدوث أضرار كبدية شديدة مرتبطة بالإجهاد التأكسدي والتغيرات الخلوية. في المقابل، يُعرف شوك الجمل بخصائصه المضادة للأكسدة والواقية للكبد. هدفت هذه الدراسة إلى تقييم السمية الكبدية الناتجة عن التعرض للحرمل من خلال دراسة تأثيره على المعايير الفسيولوجية والكيميائية الحيوية ومؤشرات الإجهاد التأكسدي لدى الجرذان البيضاء من سلالة ويستار، مع تقييم الدور الواقي للكبد الذي يمارسه شوك الجمل

تم تقسيم عشرين جرذاً أنثى إلى أربع مجموعات تجريبية: مجموعة شاهدة تلقت ماء الشرب والغذاء القياسي، ومجموعة الحرمل التي تلقت مغلي الحرمل بجرعة 60 ملغ/كغ، ومجموعة شوك الجمل التي تلقت جرعة 80 ملغ/كغ، ومجموعة الحرمل + شوك الجمل التي تلقت شوك الجمل بجرعة 80 ملغ/كغ تلاه بعد ساعة واحدة الحرمل بجرعة 60 ملغ/كغ. أُعطيت جميع المعالجات عن طريق الفم لمدة 45 يوماً متتالياً

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الإجهاد التأكسدي، الحماية الكبدية، السمية الكبدية، نبات شوك الجمل، نبات الحرمل، الكلمات المفتاحية

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Abbreviation list

ALAT: Alanine Aminotransferase
ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
ANOVA: Analysis of Variance
ASAT: Aspartate Aminotransferase
AST: Aspartate Aminotransferase
Bax: Bcl-2 Associated X protein
Bcl-2: B-cell Lymphoma 2
BCG: Bromocresol Green
BSA: Bovine Serum Albumin
C: Control Group
CAT: Catalase
CHCl₃: Chloroform
CNS: Central Nervous System
DNA: Deoxyribonucleic Acid
DTNB: 5,5'-Dithio-bis-(2-Nitrobenzoic Acid)
EDTA: Ethylenediaminetetraacetic Acid
Ethanol: C₂H₅OH
g: Gram
g/L: Gram per Liter
GSH: Reduced Glutathione
H₂O: Water
H₂O₂:HydrogenPeroxide
HSV-2:Herpes Simplex Virus Type 2
I.P.A.: Institut Pasteur d'Alger
M: Molar
MDH: Malate Dehydrogenase
mg/kg: Milligram per Kilogram

ml: Milliliter

MPO: Myeloperoxidase

MT: Milk Thistle

Na₂HPO₄: Disodium Hydrogen Phosphate

NAD⁺: Nicotinamide Adenine Dinucleotide (Oxidized form)

NADH: Nicotinamide Adenine Dinucleotide (Reduced form)

NaH₂PO₄: Sodium Dihydrogen Phosphate

NF-κB: Nuclear Factor Kappa B

nm: Nanometer

O₂: Molecular Oxygen

p: p-value

PGH: *Peganum harmala*

PHE: *Peganum harmala* Extract

pH: Potential of Hydrogen

ROS: Reactive Oxygen Species

RPM: Revolutions Per Minute

SD: Standard Deviation

SH: Sulfhydryl Group

SM: *Silybum marianum*

SME: *Silybum marianum* Extract

SSA: Sulfosalicylic Acid

STAT3: Signal Transducer and Activator of Transcription 3

TG: Triglycerides

Tris: Tris(hydroxymethyl)aminomethane

UI/L: International Unit per Liter

UV: Ultraviolet

VLDL: Very Low-Density Lipoprotein

λ_{max}: Maximum Wavelength

°C: Degree Celsius

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Introduction

Introduction

Medicinal plants have been utilized for centuries across various regions of the world for the prevention and treatment of numerous diseases, owing to their diverse biological properties, including antimicrobial, antiviral, antioxidant, anticancer, and anti-inflammatory activities. Although their use declined during the last century, sustained scientific interest remains in elucidating the mechanisms underlying their beneficial effects on human health (**Khan *et al.*, 2025**).

Among the commonly used medicinal plants, *Peganum harmala* occupies a prominent place. However, its potential toxicity is often underestimated, particularly when administered at doses exceeding recommended levels. This species is widely employed in traditional medicine systems in Morocco, Algeria, China, and Spain. Its extracts exhibit a broad spectrum of biological activities, including anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant, analgesic, cardioprotective, antitumoral, antidiabetic, histofunctional, cerebroprotective, and antiproliferative effects (**Abbas *et al.*, 2021**).

Despite its extensive traditional use, several experimental studies and clinical reports have demonstrated that high doses of *Peganum harmala* may induce severe toxicity. Reported adverse effects include hepatotoxicity, neurological disturbances, and impairment of vital functions, which may lead to fatal outcomes in certain cases. These findings emphasize that its safety profile is strongly dose-dependent (**Moshiri *et al.*, 2013**).

In contrast, *Silybum marianum* (milk thistle), a member of the Asteraceae family, is recognized for its significant medicinal and pharmaceutical value due to its richness in bioactive compounds. It has long been used in traditional medicine for the management of digestive, hepatic, and biliary disorders. These therapeutic effects are primarily attributed to silymarin, a flavonolignan complex present at high concentrations and responsible for most of its pharmacological activity (**Svobodova *et al.*, 2006**).

In this context, the following research question arises: to what extent can *Silybum marianum* extract protect the liver against toxicity induced by *Peganum harmala*? Additionally, what biological and histological alterations occur in hepatic tissue under these conditions?

The objective of this study is to investigate experimental hepatotoxicity induced by *Peganum harmala* in rats and to evaluate the hepatoprotective potential of *Silybum marianum* extract in mitigating hepatic oxidative stress and associated cellular damage.

This work is organized into three chapters: (1) a literature review, (2) materials and methods, and (3) results and discussion. Finally, a general conclusion and perspectives are presented.

Bibliographic part

Part 01

***Peganum harmala* Botanical and Pharmacological Aspects**

1.1 Origins and Traditional Uses of *Peganum harmala*

1.1.1 The origin and description of *Peganum harmala*

Peganum harmala L., commonly known as "*Harmal*" in North Africa and "*Espanol*" in Iran. It is frequently referred to "*African rue*," "*Mexican rue*," or "*Turkish rue*" in the US. The species is a member of the family Zygophyllaceae in the order Zygophyllales, which is a taxonomic group with over 250 species and about 22 genera. Release a potent smell. Opposite the leaves are solitary white flowers with green veins. The fruit is made up of globose capsules with three chambers, each of which holds a large number of angular, blackish seeds. Because of its bitter flavor, grazing animals tend to avoid the plant, but in times of pasture scarcity, they might eat it. This plant can poison any animal, but it most commonly affects dromedary camels(Mahmoudian *et al.*, 2002).



Figure 1. Leaves of the *Peganum harmala* plant (Weckesser, 2013).



Figure 02. Seeds of *Peganum harmala* (Asgarpanahet *et al.*, 2012).

1.1.2 Traditional Use of the Plant

Peganum harmala, aside from its fever, rheumatism, asthma, and vision-clearing benefits, has traditionally been used for inducing menstruation, expelling parasites, promoting sleep, acting as a mild sedative, enhancing sexual desire. Also, plant extracts are used to wake up the central nervous system and for fun. Plus, *Peganum harmala* is thought to fight bacteria, fungi, and parasites in North Africa and India (Apostolico *et al.*, 2016).

1.2 Phytochemical Composition and Pharmacological Effects of *Peganum harmala*

1.2.1 Phytochemical Composition of *Peganum harmala*

The alkaloids, flavonoids and anthraquinones are the main bioactive constituents in the phytochemistry profile of *P. harmala*.

As per literature, the chemical constituents are distributed as follows

Total Constituents: The total alkaloid content of *P. harmala* ranged from 2–5% (Bukhari *et al.*, 2008).

The principal beta-carboline alkaloids present in *P. harmala* extracts include harmaline, harmine, harmalol, harmol and tetrahydroharmine (Herraiz *et al.*, 2010).

Harmala contains several pharmacologically active alkaloids, especially in the seeds and roots. These are quinazolines, including vasicine and vasicinone, and β -carboline, including harmine, harmaline (which is the same as harmidine), harmalol, and harman. The unripe seeds have less content than the ripe seeds.

Harmaline (Harmidine). $C_{13}H_{15}ON_2$ - Initially separated by Göbel from the seeds and roots of *P. harmala*, this is the main alkaloid in this plant. It crystallizes in color faded or lightyellow prisms, and the substance is not optically active.

Vasicinone. $C_{11}H_{10}O_2N_2$ – An additional alkaloid found in *Adhatodavasica* Nees and *Peganum harmala*. The fundamental forms transparent crystals from 95% ethanol. It possesses $22-100^\circ$ ($c=0.5$ in $CHCl_3$) and the UV spectrum exhibits absorption peaks at 227, 272, 302 and 315 nm. The alkaloid produces crystalline salts using mineral acids. It is a dynamic bronchodilator (Glasby *et al.*, 1978).

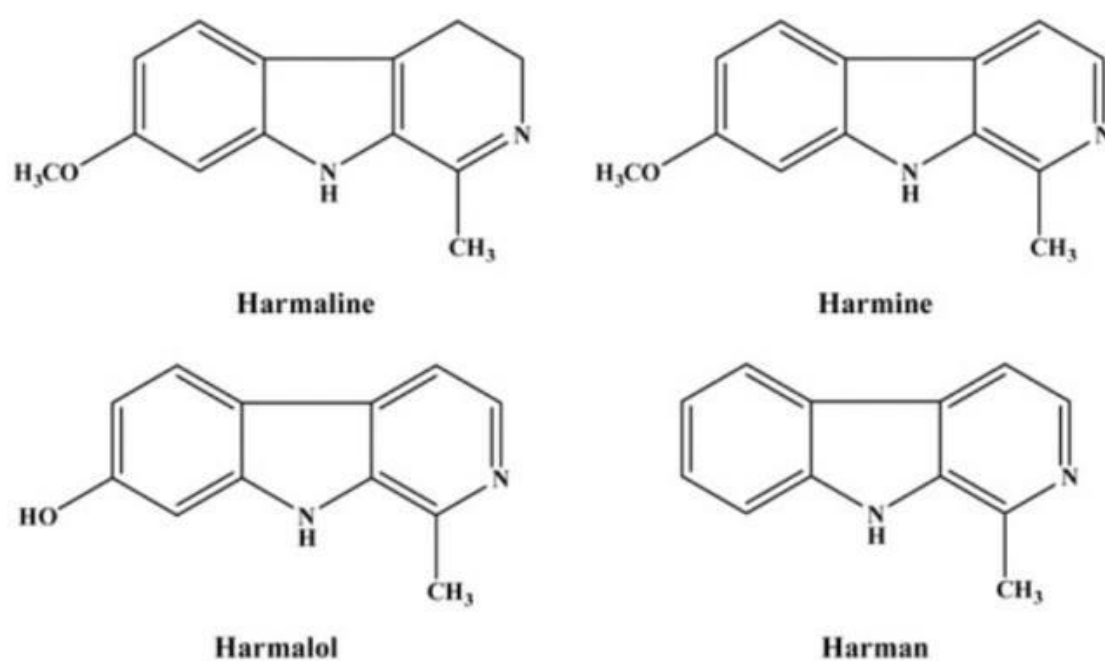


Figure 3.Phytochemical Composition of *Peganum harmala*(Mahmoudian *et al.*, 2002).

1.2.2 Pharmacological effects of *Peganum harmala*

Primarily responsible for the following traits.

1. Anti-inflammatory Effects

Peganum harmala lessens inflammation by inhibiting the myeloperoxidase (MPO) enzyme. In animal models, studies have also demonstrated that it can reduce joint swelling (**Abbas *et al.*, 2021**).

2. Antiviral Properties

Studies have shown that the methanolic extract from *Peganum harmala* seeds has virucidal qualities and works well against the type 2 herpes simplex virus (HSV-2)(**Mouloudizargari *et al.*, 2013**).

3. Cardiovascular Effects

Studies have shown that *Peganum harmala* extract reduces blood pressure, lessens cardiac hypertrophy, and enhances cardiac function in hypertensive rat models (**Mouloudizargari *et al.*, 2013**).

4. Cancer Impact

Research indicates that *Peganum harmala* combats tumors in two ways

By inducing apoptosis (programmed cell death), particularly in leukemia cells, by balancing death and survival proteins (Bax/Bcl-2), cancer cells can be directly killed by acting as an antiangiogenic drug and preventing the tumor from growing new blood vessels to receive nourishment, starvation dramatically reduces the tumor's growth and spread (**Jaradat *et al.*, 2024**).

5. Effect on Parkinson's Disease

Studies show that *Peganum harmala* reduces oxidative stress in the brain, improves motor function, and protects neurons, all of which help to lessen the symptoms of Parkinson's disease (**Razaei *et al.*, 2016**).

Part 02

Mechanisms of hepatotoxicity induced by Peganum harmala

2.1 Reported Toxicity in Animals

2.1.1 Toxic Effects of *Peganum harmala* in Clinical and Animal Studies

Several studies have investigated the toxic effects and associated syndrome of *Peganum harmala* in both in vitro and in vivo conditions. In clinical settings, the plant commonly induces bradycardia, gastrointestinal disturbances (nausea, vomiting), hallucinations, and other central nervous system (CNS) symptoms. These effects are mainly attributed to its toxic alkaloids, particularly harmaline and harmine present in the seeds (**Habbachiet *al.*, 2013**).

In animals, toxicity is characterized initially by recumbency, then shows excessive saliva production, vomiting, and diarrhea. The most noticeable signs affect the nervous system and include restlessness, muscle shaking, stiffness, loss of coordination, and rapid breathing, which may later lead to an inability to stand. Later symptoms involve respiratory distress, mydriasis, polyuria, and hypothermia, with frequent cases of abortion. Neurological manifestations are generally short-lived, and death typically occurs within 30 to 36 hours after the onset of CNS symptoms. Chronic poisoning in cattle presents with reduced appetite, persistent agitation, hind limb weakness, and joint-related sounds, particularly at the fetlock (**Mahmoudian *et al.*, 2002**).

2.2.Oxidative Stress

2.2.1.Definition

Oxidative stress refers to a physiological condition arising from an imbalance between the production of free radicals—particularly reactive oxygen species (ROS)—and the organism's capacity to neutralize them through its antioxidant defense systems. While the generation of ROS is a normal and essential process involved in cellular signaling and homeostasis, their excessive accumulation in the absence of adequate protective mechanisms leads to oxidative damage and cellular dysfunction (**Sorg, 2004**).

2.2.2.Free radicals

A free radical is a chemical entity that contains an unpaired electron in its outermost orbit. These are unstable and react quickly with any element, attempting to attain stability by acquiring an electron. A chain of reactions begins as a free radical attacks the nearest molecule by gaining an electron from that molecule, thus transforming that molecule into a free radical (**Zweier and Hassan Talukder, 2006**).

Peganum harmala alkaloids can induce oxidative stress and tissue damage when administered at high doses, as shown in several experimental studies in rats, with an increase in liver enzymes and histopathological alterations of the liver .

2.3 Hepatocellular Injury, Inflammation, and Apoptosis

2.3.1 Definition of hepatotoxicity

Hepatotoxicity refers to the potential of a substance to induce liver injury, regardless of the underlying mechanism.

This toxicity may manifest in different forms, including acute or subacute presentations (Wallace *et al.*, 2010 ; Berthélémy, 2015)

2.3.2 Definition of Inflammation

Inflammation is a physiological response that occurs as a reaction to injury caused by physicochemical factors like irradiation, burns, etc., or as a response to infections like bacterial, viral, or parasitic infections.

Inflammation is generally a beneficial response to injury or infection. Its purpose is to activate the immune response to eliminate the noxious agent and repair tissue damage (Medzhitov, 2008).

2.3.3 Apoptosis

Apoptosis or programmed cell death occurs during the normal formation of tissues or as a result of cellular damage and tumor formation. Apoptosis or programmed cell death is a series of distinct morphological changes that occur in a dying cell, including thickening of the cell's cytoplasm and nucleus, followed by disruption of the cell into membrane-bound apoptotic bodies that contain a variety of cellular structures and nuclear components, which are then phagocytosed by adjacent cells or macrophages (Hamsa *et al.*, 2011).

- *Peganum harmala* may cause a severe tissue damage, which included liver injury, inflammation, cell necrosis (Benbottet *et al.*, 2022) .

Part 03

Medicinal plants with hepatoprotective effects (*Silybum marianum*)

3.1 Natural Antioxidants and the Liver

3.1 Antioxidants and Plant-Based Antioxidant Compounds

Antioxidants are substances that can slow down or prevent the formation of harmful oxidant molecules, neutralize those already present, and help remove them, thereby stopping oxidative chain reactions. In nature, especially in plants, many bioactive compounds have strong antioxidant properties. Various plant species, such as *Citrullus colocynthis*, *Limoniastrum feei*, and *Pistacia lentiscus*, are known for their antioxidant activity. This activity is mainly due to natural compounds commonly found in foods, including polyphenols, phenolic acids, carotenoids, flavonoids, and tannins (Dai & Mumper, 2010).

3.1.2 Definition of the plant

Silybum marianum, also called milk thistle, is an edible plant belonging to the Asteraceae family. It is an annual or every two years plant native to the Mediterranean regions of Europe, North Africa, the Middle East, and some regions of the USA. The plant grows to a height of three to ten feet with a stem supporting large, alternating, spiky-edged leaves.

Its common name, milk thistle, is derived from the white veins on the leaves, which ooze a milk-like sap when torn. Each stem bears a single, large, purple flower with pointed spines at the end. The fruit section of the plant is shiny brown or dull with blemishes (Porwal *et al.*, 2019).



Figure 4. Milk Thistle Plant (*Silybum marianum*) in Bloom (LPEV, s.d.).



Figure 5. Raw Seeds of *Silybum marianum*(Abenavoliet *et al.*, 2018).

Silybum marianum exhibit hepatoprotective, anticancer, antioxidant, anti-inflammatory, hypoglycemic, neuroprotective, cardioprotective, and immunomodulatory effects (Karimzadeh *et al.*, 2024; Ma *et al.*, 2024)

3.2 pharmacological effects

Effect on liver

Silymarin protects liver cells by stabilizing their membranes. It reduces lipid peroxidation, which helps maintain membrane fluidity and integrity. It also blocks the binding of certain mycotoxins, such as phalloxin peptides and α -amanitin, to receptors on liver cell membranes. As a result, membrane damage is reduced, the entry of toxins into the cells is limited, and the recycling of toxins between the liver and intestine is decreased, which increases the resistance of liver cell membranes (Saller *et al.*, 2008; Abenavoliet *et al.*, 2010).

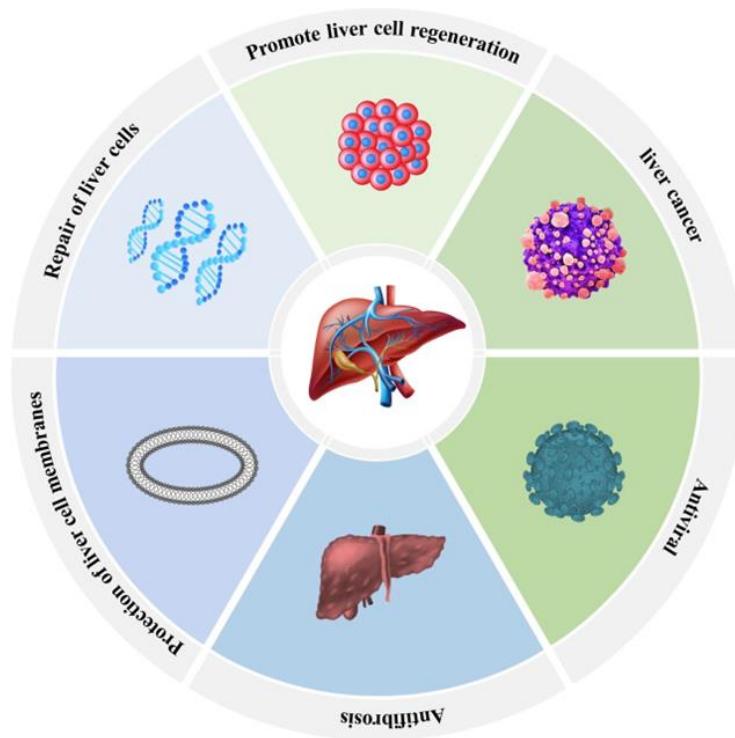


Figure 6. Therapeutic Effects of *Silybum marianum* on Liver Diseases (Zhang *et al.*, 2024).

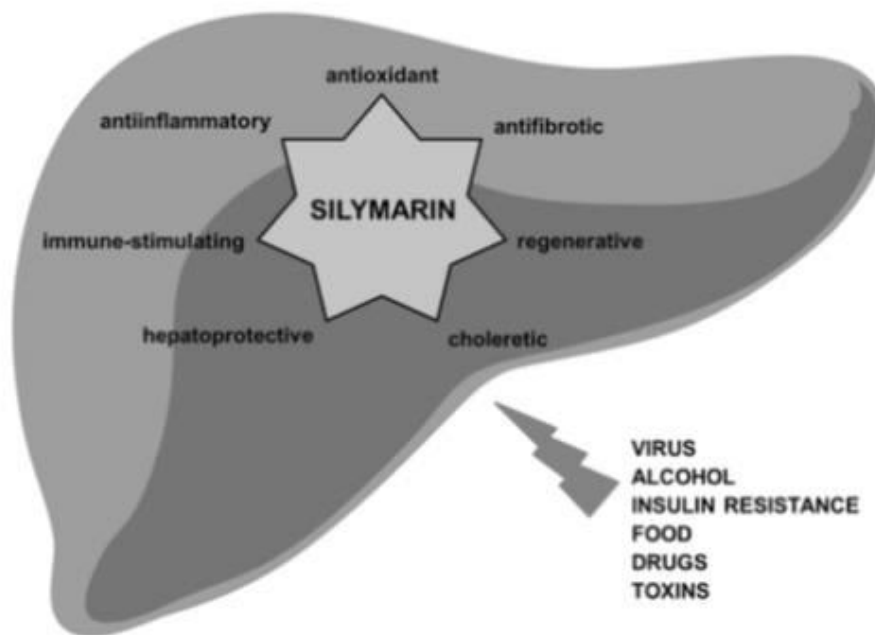


Figure 7. Pharmacological Effects of Silymarin on the Liver (Abenavoliet *et al.*, 2018).

Anticancer effect

Silymarin can reduce both the size and number of lung cancer cells due to its anti-angiogenic activity. This effect is linked to a decrease in cytokine production by tumor-associated macrophages and the inhibition of NF- κ B and STAT3 activation in lung cancer cells (**Tyagi et al., 2009**). In addition, *silymarin* has been reported to inhibit the growth of SN12K1 kidney cancer cells (**Hii et al., 1998**).

Anti-inflammatory effect

The anti-gastric ulcer effect of *silymarin* is related to its ability to inhibit lipid peroxidation in the lipoxygenase pathway, which reduces leukotriene synthesis (**Alarcon de la Lastra et al., 1992**). *Silymarin* also acts as a free radical scavenger. In addition, it regulates the arachidonic acid pathway and decreases the production of prostaglandins and leukotrienes, which helps limit the development of arthritis (**Gupta et al., 2000**).

Neuroprotective effect:

Silymarin may act as a neuroprotective agent and could be useful in managing neurological disorders such as Alzheimer's disease, Parkinson's disease, and cerebral ischemia (**Borah et al., 2013**).

3.3 Chemical compositions

Silymarin is an established dry extract of MT seeds, which are rich in flavonolignans (70% to 80% w/w), along with polymeric and oxidized polyphenolic derivatives, which include flavonoids. Flavonolignans, which are derived for the first time from the seeds of *Silybum marianum*, are a minor group of compounds, where the flavonoid part of the molecule is coupled with lignans. The flavonolignans present in *silymarin* include silybin, isosilybin A and B, silydianin, and silychristin.

The achene of *milk thistle* contains 15 to 30% fatty acids, of which about 60% is linoleic acid, 30% oleic acid, and 9% palmitic acid; roughly 25 to 30% proteins (including tyramine and betaine); along with tocopherol (approximately 0.038%), phenolic derivatives, and sterols (about 0.063%) like campesterol, stigmasterol, and sitosterol. Certain sugars (arabinose, rhamnose, xylose, glucose) along with mucilage are also found. (**Abenavoliet al., 2018**).

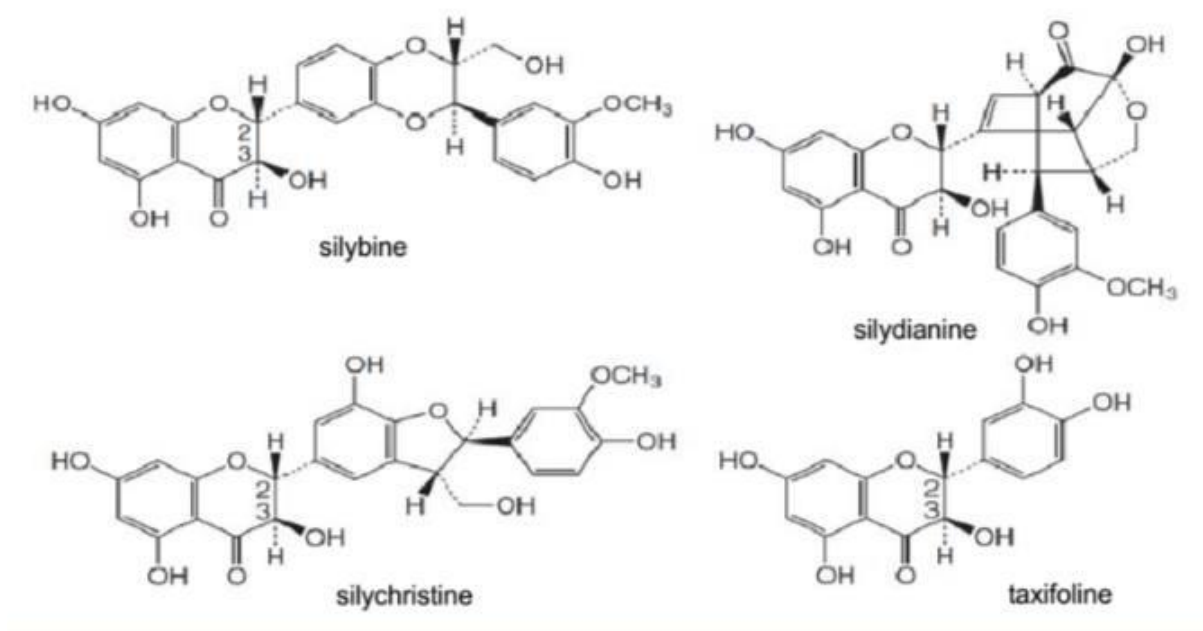


Figure 8. Structures of the main constituents of silymarin (Müzeset *al.*, 1990).

Experimental Part

Materials and Methods

3. Materials and Methods

Our study was conducted in the Biochemistry Laboratory of the Department of Applied Biology at the Faculty of Exact Sciences and Natural and Life Sciences, University of Tebessa, with the objective of investigating the experimental hepatotoxicity induced by *Peganum harmala* in rats and evaluating the hepatoprotective potential of Milk Thistle(*Silybum marianum*) extract in mitigating hepatic oxidative stress and cellular damage.

3.1. Biological materials

3.1.1 Plant materials

Our research concentrates on two plants exhibiting divergent biological activities: *Peganum harmala* (PGH) was studied as a potential source of hepatotoxicity, while *Silybum marianum* (SM) is acknowledged for its hepatoprotective qualities and effectiveness in reducing liver damage.

3.1.1.1 Source and Storage of Plants

We acquired both species (*Peganum harmala* and *Silybum marianum*) as dried plants from a local herbalist in Tebessa. We cleaned the seeds and dried them at 40 °C if necessary we ground them finely. We sieved to obtain a homogeneous powder and stored them in the right way.



Figure 9. Preparation of the plant material: Precise weighing of powders and storage in specialized airtight bags (**personal photos**).

3.1.1.2 Preparation of the infusion

We weighed 10 g of the powder and heated 100 mL of distilled water to 90 °C. Then we added the powder to the hot water, covered it immediately, and allowed it to infuse for 20 minutes (for *Peganum*) and 30 minutes (for *Silybum*) with gentle agitation. The mixture was filtered using Whatman No. 1 filter paper and then left to cool at room temperature.

3.1.1.3 Care and treatment of the animals

3.1.1.3.1 Animals and Housing Conditions

For this study, twenty (20) female Wistar rats , aged five weeks, were obtained from the Pasteur Institute of Algiers (I.P.A.). The rodents were bred and maintained at the animal facility of Larbi Tebessi University, Tebessa. The rats lived in polyethylene cages that had

important information on them, like batch number, treatment information, and experimental timelines.

The cages had sterilised wood shavings as bedding, and they were changed every day to keep the cages as clean as possible.

3.1.1.3.2 Acclimatisation and Environmental Conditions

Before the experiments started, the rats had to get used to their new home for two weeks.

During this period, environmental conditions were scrupulously regulated, maintaining a room temperature of $22 \pm 2^\circ\text{C}$ and a natural 12-hour light/dark cycle to emulate physiological circadian rhythm



Figure10. Rat housing and acclimatization environment (**Personal photos**).

3.1.1.3.3 Diet and Hydration

The animals were given a standard, nutritionally complete pellet diet and always had access to fresh drinking water to make sure they stayed hydrated.

Table 01. composition of 1 kg of food.

	Quantity in g/kg of food	%
Protein	140-180	14-18
Grains	500-600	50-60
Fats	40-80	4-8
Fiber	80-120	8-12
Minerals&Vitamins	10-30	1-3
Veggies&Herbs	50-100	5-10
Extras	5-10	0.5-1

3.2. Methods

3.2.1 Experimental Design and Treatment

After the acclimatisation period, at the commencement of the experimental protocol, the rats exhibited an average initial body weight of 200 grams, the rats were divided into four groups: three treatment groups and one control group. Each group had five rats. All treatments were given over a period of 45 days in a row.

Table 02 .The Experimental design of the study.

Experimental Groups	Treatment
Control	Received distilled water by oral gavage.
PGE	Received drinking water and a specific dose of a decoction prepared from 60 mg/kg of <i>Peganum</i> via oral gavage.
SME	Received drinking water and a specific dose of a decoction prepared from 80 mg/kg of <i>Silybum</i> via oral gavage.
PGE + SME	Received <i>Silybum</i> (80 mg/kg body weight), and after 1 hours Received <i>Peganum</i> (60 mg/kg body weight) by oral gavage.

Every week during the experiment, the body weight of both the treated and untreated rats was measured. Also, each rat's daily weight was checked to make sure that the right amount of medication was given to each one based on its own weight.



Figure 11. Rat weighing and oral gavage administration steps **(Personal photos)** .

3.2.2 Blood sampling

3.2.2.1. Sacrifice

Following a 45 -day treatment period, the rats decapitation was executed with a sharp blade, and arterial-venous blood was collected in heparinized tubes, which were clearly labeled for group identification.



Figure 12. Rat euthanasia, blood collection, and dissection steps (**Personal photos**)

3.2.2.2. Sample Processing

The blood samples were then transported to the laboratory in temperature-controlled coolers to maintain their integrity. Afterward, the samples underwent centrifugation at 3,000 RPM for 20 minutes to separate the plasma



Figure 13. Rat organ harvesting, weighing, and preservation steps **(Personal photos)**

3.2.2. 3.Biochemical Analyses

Plasma samples are assessed for key biochemical parameters, including

Blood Glucose Measurement Procedure:

The blood glucose measurement in rats is performed directly and accurately through the following steps: The first step involves safely restraining and handling the rat to control its movement and minimize stress. This is followed by pricking the rat's tail using a sterile needle to stimulate the emergence of a blood drop. In the final step, the blood drop is applied to the test strip connected to the digital glucometer to obtain the immediate reading displayed on the screen.



Figure 14. Rat blood glucose measurement steps **(Personal photos)**.

Liver function markers

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin.

Lipid profile

Triglycerides

3.2.3. Liver tissue sampling

A longitudinal abdominal incision was performed to excise the liver. Following dissection, adherent adipose tissue was carefully removed, and the organs were weighed.

Subsequently, the liver samples were stored at -20°C in a cryogenic freezer to preserve their biochemical integrity pending further analysis of oxidative stress biomarkers, including the

estimation of reduced glutathione (GSH) levels and the determination of catalase enzyme activity.

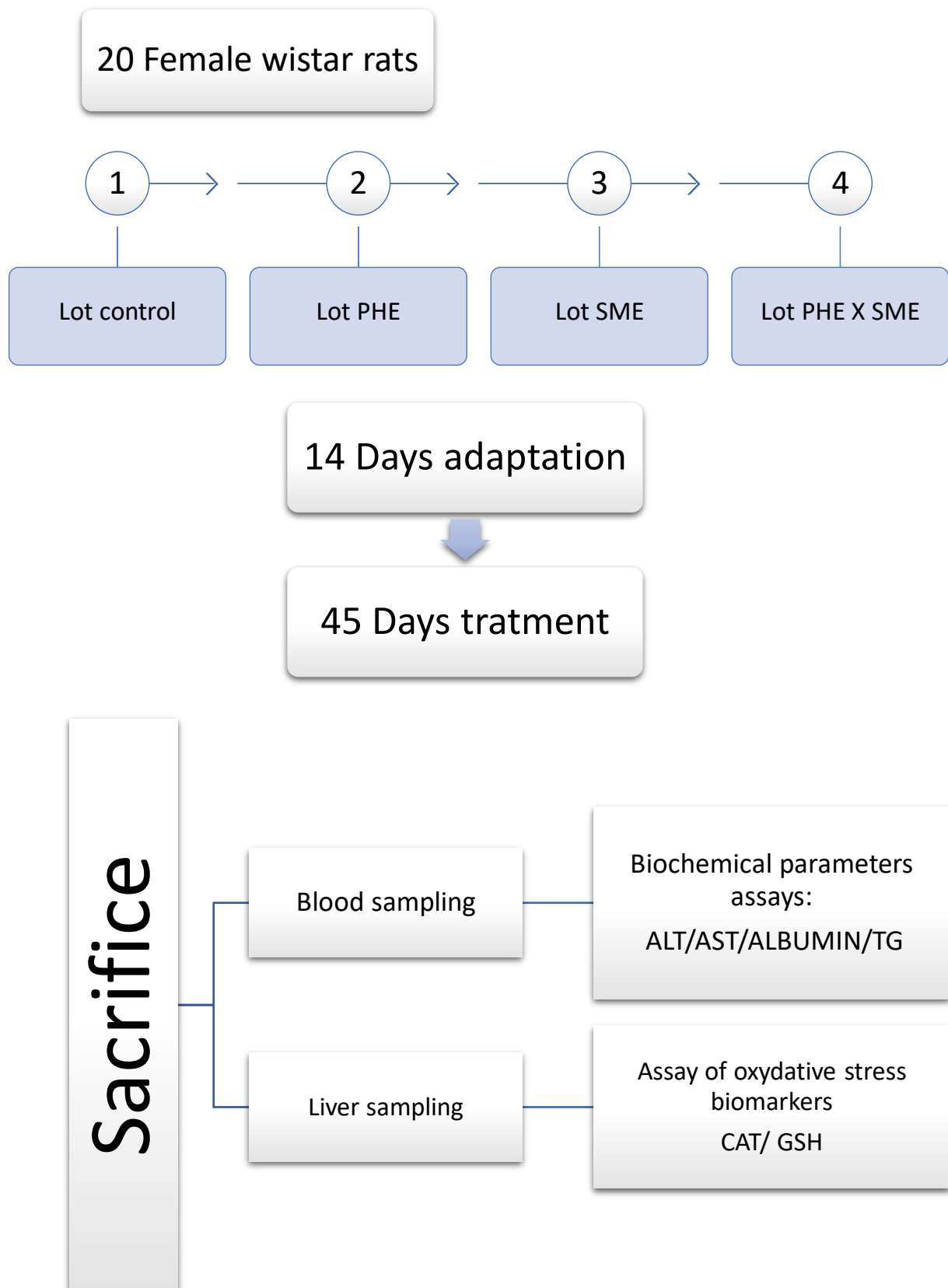


Figure 15. Diagram summarizing the experimental protocol.

3.2.4 .Albumin Assay

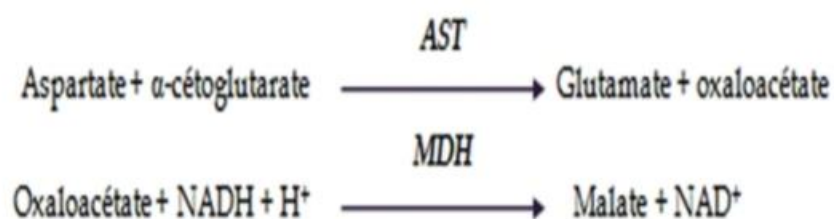
Principle

In a buffered aqueous solution maintained at pH 4.2, bromocresol green (BCG) undergoes a selective binding interaction with albumin, resulting in the formation of a chromogenic complex. The absorbance of this complex exhibits a linear relationship with albumin concentration when measured spectrophotometrically at its λ_{max} of 630 nm (with an acceptable measurement range of 620-640 nm). This photometric property serves as the basis for quantitative albumin determination in biological specimens (**Doumas *et al.*, 1997**).

3.2.5. Triglycerides Assay Principle The assay is based on an **enzymatic** colorimetric method in which triglycerides are enzymatically hydrolyzed to produce glycerol and free fatty acids. The glycerol is subsequently oxidized, leading to the generation of a colored chromophore through a series of coupled enzymatic reactions. The intensity of the resulting colorimetric signal, measured spectrophotometrically, is directly proportional to the concentration of triglycerides in the sample, enabling quantitative analysis (**Parhofer and Laufs, 2019**).

3.2.6. Assay of Aspartate amino transferase AST Principle

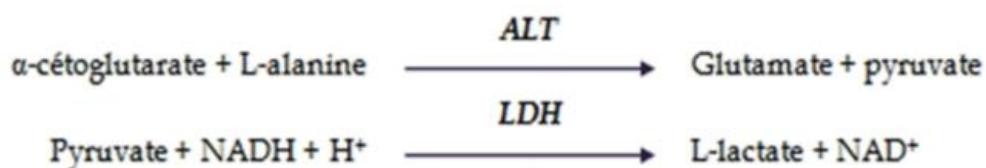
The principle is shown in the following reaction diagram



The spectrophotometric assay of Aspartate Aminotransferase (AST) quantifies enzymatic activity by monitoring the transamination of L-aspartate and α -ketoglutarate to yield oxaloacetate and L glutamate. This reaction is coupled with the oxidation of NADH to NAD^+ , catalyzed by malate dehydrogenase (MDH), wherein the decrease in absorbance at 340 nm—proportional to NADH consumption—serves as a direct measure of AST activity. The assay thus provides a kinetic assessment of enzyme function through the stoichiometric relationship between substrate depletion and cofactor conversion (**Rosalki and Rau, 1972**).

3.2.7. Assay Of Alanine amino transferase ALT Principle

The principle is shown in the following reaction diagram



The alanine aminotransferase (ALT) assay quantitatively measures enzymatic activity through spectrophotometric analysis by monitoring the transamination reaction between alanine and α ketoglutarate, which yields pyruvate and glutamate as products. This reaction is coupled with the reduction of nicotinamide adenine dinucleotide (NAD⁺) to NADH, enabling the kinetic assessment of ALT activity based on absorbance changes at 340 nm (**Bradford, 1976**).

3.3.8. Oxidative stress analyses

3.3.8.1 Assay of Catalase (CAT) Activity Principle

Catalase activity was determined following the method described by **Aebi (1984)**. The assay is based on measuring the decrease in optical density (absorbance) at 240 nm resulting from the decomposition of hydrogen peroxide (H₂O₂) by the enzyme. The reaction is carried out at 25°C using a spectrophotometer. Absorbance readings are recorded after an initial 15-second delay and monitored over a total duration of 60 seconds. Due to the rapid decline in catalase activity, the time interval between pipetting and placing the cuvette into the spectrophotometer must be strictly kept identical for all samples.

Preparation of the Tissue Homogenate

100 mg of tissue sample was homogenized in 1 mL of phosphate buffer (0.1 M, pH 7.0).

Homogenization

The mixture underwent cold grinding to obtain the raw homogenate.

Centrifugation

The resulting homogenate was centrifuged at 9000 rpm for 15 minutes to separate the cytosolic fraction (supernatant) used for subsequent enzymatic assays.

Operating Procedure

The reaction mixture was prepared in spectrophotometric cuvettes according to the following volumes:

Blank Tube

Phosphate Buffer (0.1 M, pH 7.0): 800 μ L

H₂O₂ Solution (0.5 M): 200 μ L

Supernatant (Enzymatic Source): 0 μ L (None)

Sample Tube

Phosphate Buffer (0.1 M, pH 7.0): 780 μ L

H₂O₂ Solution (0.5 M): 200 μ L

Supernatant (Enzymatic Source): 20 μ L

Calculation:

The activity of catalase is determined using the following equation:

$$\text{Catalase Activity } (\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}) = (\Delta A \times 10) / (\epsilon \times L \times X \times \text{Fd})$$

Where:

- ΔA : Change in absorbance per minute
- ϵ : Extinction coefficient of H₂O₂ = 0.04 mM⁻¹·cm⁻¹
- L: Path length of the cuvette = 1 cm
- X: Protein concentration in mg/mL
- Fd: Dilution factor of H₂O₂ in the buffer (0.02)

This equation quantifies the enzyme's ability to decompose hydrogen peroxide (H₂O₂) per minute per milligram of protein.

3.3.8.2 Assay of Reduced Glutathione (GSH) Content

Principle

Reduced glutathione content was quantified according to the method described by **Weckbeker and Cory (1988)**. The assay is based on the reduction of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) by the sulfhydryl (-SH) groups of GSH, yielding a yellow-colored product, 2-nitro-5-mercapturic acid, which exhibits an absorption peak at 412 nm. Prior to the

reaction, a deproteinization step is required to exclude non-specific protein thiols and isolate the specific GSH fraction.

Preparation of the Tissue Homogenate

100 mg of tissue sample + 4 mL of EDTA solution (0.02 M).

Procedure

The tissue was subjected to cold grinding, and the homogenate was collected.

Operating

Procedure

1. Transfer 0.8 mL of the raw tissue homogenate into a clean tube.
2. Precipitate proteins by adding 0.2 mL of the 0.25% sulfosalicylic acid (SSA) solution.
3. Agitate the mixture thoroughly and incubate in an ice bath for 15 minutes.
4. Centrifuge the mixture at 1000 rpm for 5 minutes to pellet the precipitated proteins.
5. Collect 0.5 mL of the clear supernatant.
6. Add 1 mL of Tris-EDTA buffer (pH 9.6).
7. Mix well, then add 0.025 mL of the 0.01 M DTNB solution.
8. Incubate for 5 minutes at room temperature to allow complete stabilization of the yellow color that develops instantaneously.
9. Measure the absorbance at 412 nm against an appropriate reagent blank.

Calculation:

The glutathione concentration was calculated using the following formula:

$$[\text{GSH}] (\text{nM GSH/mg protide}) = (\text{DO} \times 1 \times 1,525) / (13100 \times 0,8 \times 0,5 \times \text{mg protide})$$

Where:

- **OD:** Optical density at 412 nm.
- **1:** Total volume of solutions used in deproteinization (0.8 mL homogenate + 0.2 mL SSA).
- **1.525:** Total volume of solutions used in the GSH assay (0.5 mL supernatant + 1 mL Tris-EDTA + 0.025 mL DTNB).
- **13,100:** Extinction coefficient of the thiol group (–SH) at 412 nm.
- **0.5:** Volume of supernatant present in the 1.525 mL total assay volume.

- **0.8:** Volume of homogenate present in the 1 mL total deproteinization volume.

3.3.8.3 Quantification of Total Proteins

Principle

Total protein concentration was determined using the **Bradford (1976)** method. This colorimetric assay relies on the binding of Coomassie Brilliant Blue G-250 dye to proteins under acidic conditions. The dye interacts primarily with arginine and, to a lesser extent, with histidine, lysine, and aromatic residues through non-covalent forces (hydrogen bonds, hydrophobic, and ionic interactions). Upon protein binding, the absorbance maximum of the dye shifts from 465 nm (red form) to 595 nm (blue form).

Operating Procedure

1. Aliquot 0.1 mL (100 µL) of the sample supernatant into a test tube.
2. Add 4 mL of the Bradford reagent.
3. Vortex the mixture thoroughly and allow it to incubate at room temperature for 5 minutes to stabilize the color.
4. Measure the optical density at 595 nm against a blank containing 4 mL of Bradford reagent and 0.1 mL of distilled water.

The protein concentration of the samples was calculated by referencing the absorbance values against a standard calibration curve plotted using Bovine Serum Albumin (BSA, 1 mg/mL) under identical conditions.

3.3. Preparation of Bradford Reagent (Coomassie Brilliant Blue Solution)

Dissolve 50 mg of Coomassie Brilliant Blue G-250 in 25 mL of 95% ethanol. The beaker must be covered with aluminum foil.

Stir the solution continuously for 2 hours.

Add 50 mL of 85% orthophosphoric acid, then bring the final volume to 500 mL with distilled water.

Filter the solution and store at 4°C in the dark (maximum shelf life: 1 month).

Preparation of the BSA Standard Calibration Curve

BSA Stock Solution

Dissolve 1 mg of BSA in 1 mL of distilled water under constant agitation.

Standard Range Set-up

Prepare 6 tubes with varying compositions as follows, mix well, and record absorbance at 595 nm:

Tube 1 4 mL Bradford Reagent + 100 μ L Distilled Water + 0 μ L BSA Stock

Tube 2 4 mL Bradford Reagent + 80 μ L Distilled Water + 20 μ L BSA Stock

Tube 3 4 mL Bradford Reagent + 60 μ L Distilled Water + 40 μ L BSA Stock **Tube 4** 4 mL Bradford Reagent + 40 μ L Distilled Water + 60 μ L BSA Stock

Tube 5 4 mL Bradford Reagent + 20 μ L Distilled Water + 80 μ L BSA Stock

Tube 6 4 mL Bradford Reagent + 0 μ L Distilled Water + 100 μ L BSA Stock

The resulting standard curve is used to determine the linear regression equation ($y = ax + b$) for calculating protein concentration in unknown samples.

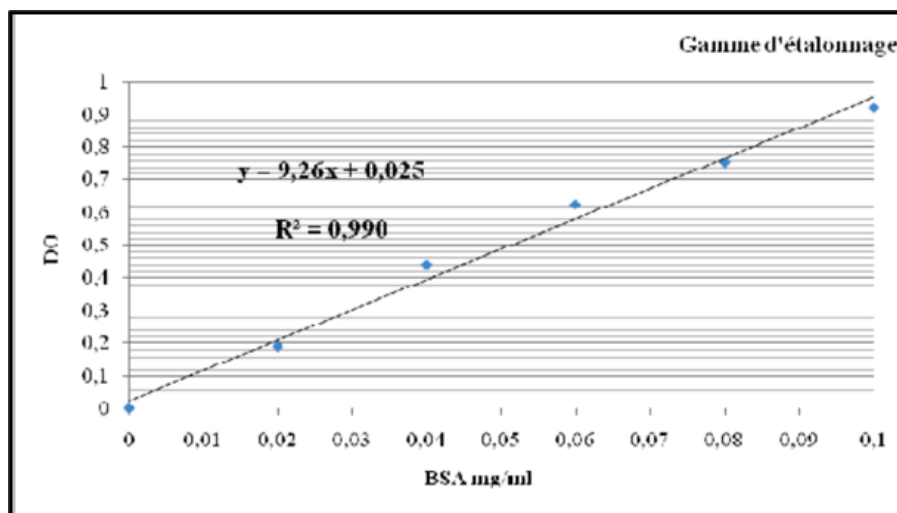


Figure16.Bovineserumalbumincalibrationcurve



Figure 17.Steps of oxidative stress assays (**Personal photos**).

Comparative Statistical Evaluation of Experimental Groups
A statistical analysis was performed to compare the effects of the different treatments among the experimental groups

control (C), *Silybum marianum* extract-treated group (SME), *Peganum harmala* extract-exposed group (PGE), and the co-treated group (SME+PGE). Data were expressed as mean $\pm$ standard deviation (SD), and one-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance. Differences were considered statistically significant at $P < 0.05$. This analysis revealed significant variations between groups, allowing for the evaluation of the biological effects and potential interactions between the *Silybum marianum* and *Peganum harmala* extracts.

Results and Discussion

4. Results and Discussion

4.1. Physiological parameters

4.1.1. Physiological parameters Effect of various treatments on body weight

Administration of *Peganum harmala* extract (PHE) affected body weight evolution throughout the experimental period. Rats treated with PHE showed irregular weight gain compared with the other groups, suggesting a systemic toxic effect associated with the extract. In contrast, co-administration of *Silybum marianum* extract (SME) with PHE improved body weight progression and maintained a more stable growth pattern. The results revealed a clear and continuous decrease in body weight in the group treated with Camel Thistle extract (SME) compared to the control group (C). This reduction began from the first week and persisted throughout the experimental period, confirming the body-weight-lowering effect exerted by the SME extract. These findings suggest that SME may attenuate the negative physiological effects induced by PHE.

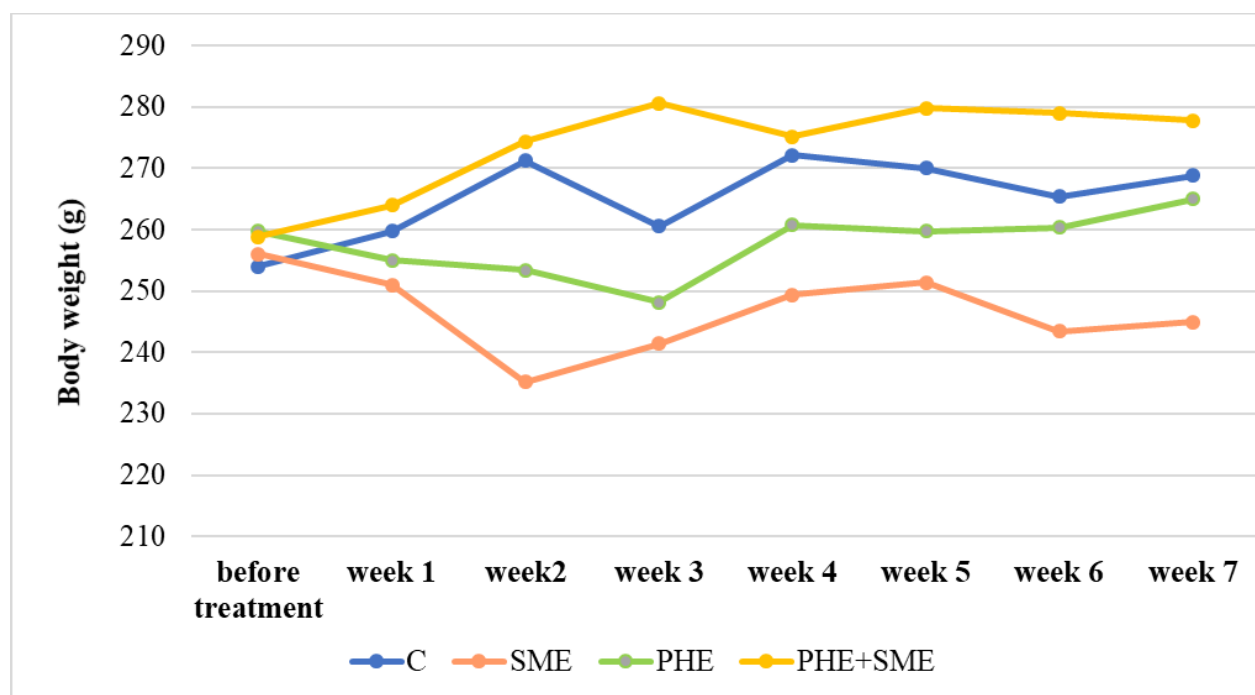


Figure 18. Curve showing the evolution of body weight (g) in the different experimental groups during the treatment period.

Research has demonstrated that repeated oral administration of *P. harmala* alkaloid extracts in rats induced signs of toxicity associated with reduced body weight progression and physiological disturbances (Wang *et al.*, 2019). The improvement in body growth observed after SME co-administration may be attributed to the protective properties of *Silybum marianum*, particularly its antioxidant flavonolignans, which are known to preserve cellular integrity and reduce oxidative stress-induced damage (Mahliet *et al.*, 2015).

The body-weight-lowering effect observed with (SME) is attributed to silymarin, the primary active constituent of *Silybum marianum*. This finding is consistent with previous studies demonstrating silymarin possesses anti-obesity and anti-adipogenic properties by regulating key transcription factors involved in adipogenesis (Zhu *et al.*, 2014), alongside its role in improving insulin sensitivity and reducing leptin resistance, which directly contributes to suppressing weight gain (Guo *et al.*, 2016).

4.1.2 Effect of treatment on liver weight The administration of *Peganum harmala* extract (PHE)

The administration of *Peganum harmala* extract resulted in a significant increase in both absolute and relative organ weight, indicating possible hepatotropic effects and tissue alterations induced by the extract. In contrast, *Silybum marianum* extract (SME) alone did not produce marked changes in organ weight. However, co-administration of SME with PHE attenuated these alterations and reduced organ weight compared with the PHE-treated group. These findings suggest that although SME alone did not exhibit significant hepatomodulatory effects, it exerted a protective action against PHE-induced hepatic alterations, highlighting its potential hepatoprotective activity against toxic liver injury.

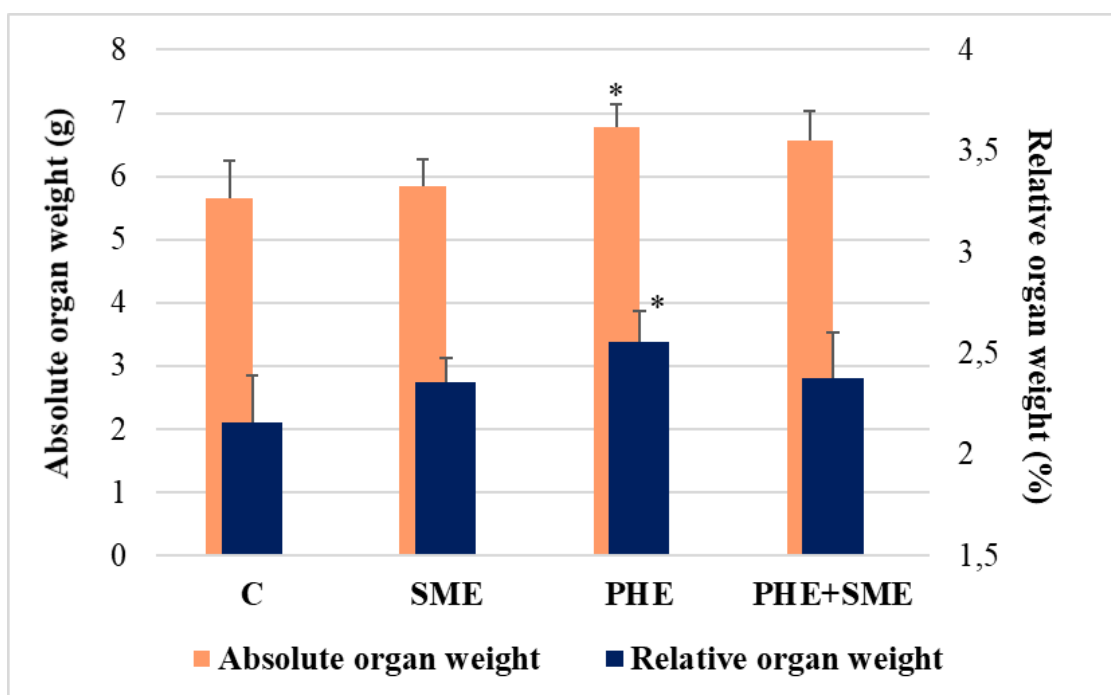


Figure 19. Bar chart representing absolute (g) and relative (%) organ weights in the different experimental groups.

Similar findings were reported in experimental studies demonstrating that prolonged exposure to *Peganum harmala* induced alterations in liver morphology and organ weight associated with hepatotoxicity (Lamchouri et al., 2002; Wang et al., 2019). In contrast, treatment with *Silybum marianum* extract alone did not induce marked variations in liver weight, indicating the absence of toxic effects on hepatic tissue. However, co-administration of *Silybum marianum* with *Peganum harmala* attenuated these alterations and restored liver weight toward normal values compared with the *Peganum* treated group. These observations are consistent with previous studies showing that silymarin exerts hepatoprotective effects through its antioxidant and membrane-stabilizing properties, reducing hepatic enlargement and tissue injury in toxicological models (Mahli et al., 2015; Eminzadeet al., 2008). Therefore, the present findings suggest that Silymarin may exert a protective effect against *Peganum* induced hepatic alterations and could be considered a potential natural hepatoprotective agent.

4.2. Biochemical parameters

4.2.1 Effect of treatment on blood glucose levels

The present results showed that administration of *Peganum harmala* extract (PHE) did not induce major alterations in blood glucose levels, suggesting that the hepatotoxic effects observed in treated rats were not directly associated with significant disturbances in carbohydrate metabolism.

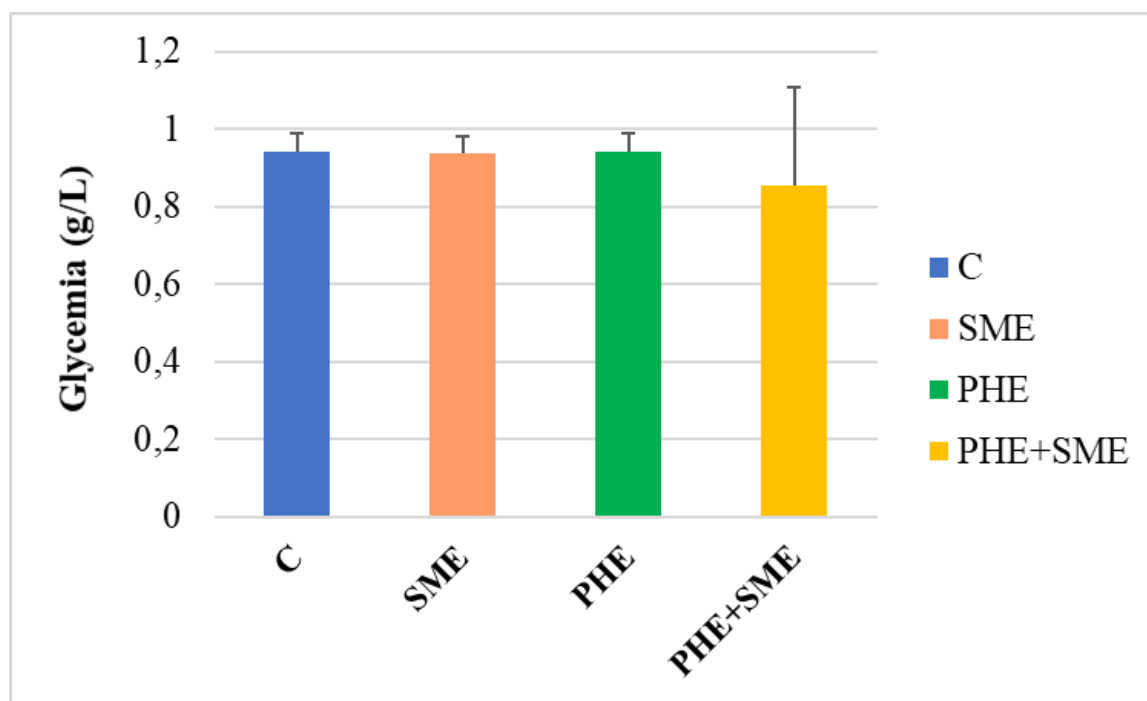


Figure 20. Bar chart representing blood glucose levels (g/L) in the different experimental groups.

Glycemia levels remained relatively stable among the experimental groups, with no major disturbances. Overall, these results indicate that the treatments did not induce significant alterations in glucose metabolism under the experimental conditions.

Similar observations were reported by **Lamchouriet al. (2002)**, who demonstrated that chronic exposure to *Peganum harmala* produced toxic effects without significantly affecting glycemic status. However silymarin may exert a protective and supportive effect against diabetes through its antioxidant properties (**Soto et al., 2003**).

Treatment with PHE resulted in significant decreased triglyceride levels, a similar reduction was observed in the SME-treated group in comparison with the control group. Whereas the combined treatment partially restored triglyceride concentrations.

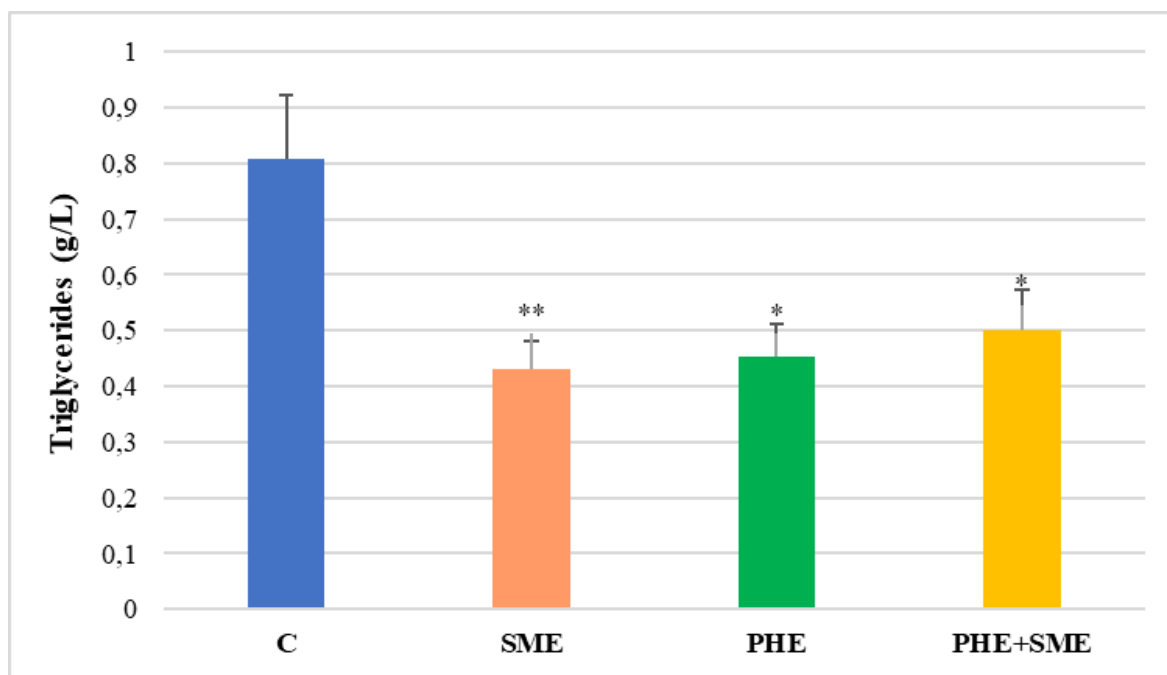


Figure21. Plasma triglycerides concentration (g/L) in the different experimental groups (*P<0,05 **P<0,01 relative to the control group (C)) .

Lipid esters consisting of glycerol bound to three fatty acids that serve as the primary energy storage reservoir, transported in blood via VLDL and chylomicrons (**Burtis & Bruns, 2014**).

The underlying mechanisms driving the decrease in triglyceride levels appear to differ depending on the specific extract. On one hand, the effect observed in the PHE-treated group is likely attributable to the presence of harmaline. According to **Li et al. (2020)**, this compound reduces lipid accumulation and induces browning in 3T3-L1 adipocytes, thereby contributing to an anti-obesity effect. On the other hand, the reduction in triglyceride levels induced by SME follows a distinct pathway, closely linked to the regulation of the gut microbiota and the subsequent production of vitamin B12 by associated bacteria (**Sun et al., 2023**).

Silymarin has been reported to reduce lipid peroxidation and improve hepatic lipid metabolism by stabilizing hepatocyte membranes and scavenging free radicals. Similar findings were reported by **Okiljević et al. (2024)**, who demonstrated that silymarin administration improved biochemical disturbances associated with oxidative liver injury.

In addition, **Abdel-Moneim et al.(2015)** demonstrated that silymarin exerted significant hepatoprotective effects by improving lipid metabolism and reducing oxidative stress-induced hepatic injury.

4.2.3. Effect of treatment on alanine aminotransferase(ALT) and aspartate aminotransferase (AST)

Regarding hepatic biomarkers, a significant increase in both ALT and AST activities was observed in the PHE-treated group ($P < 0.01$) compared to the control group (C). This joint elevation reflects distinct hepatocellular injury and membrane destabilization induced by *Peganum harmala* extract intoxication, likely driven by oxidative stress mechanisms. In contrast, the group treated with SME alone exhibited a significant decrease in the baseline activities of both transaminases. Notably, the co-administration of SME alongside PHE successfully attenuated the elevation of these enzymes, resulting in a highly significant reduction in AST levels ($P < 0.001$). This marked suppression of both ALT and AST leakage firmly demonstrates the potent hepatoprotective effect of SME against *Peganum harmala*-induced hepatic damage

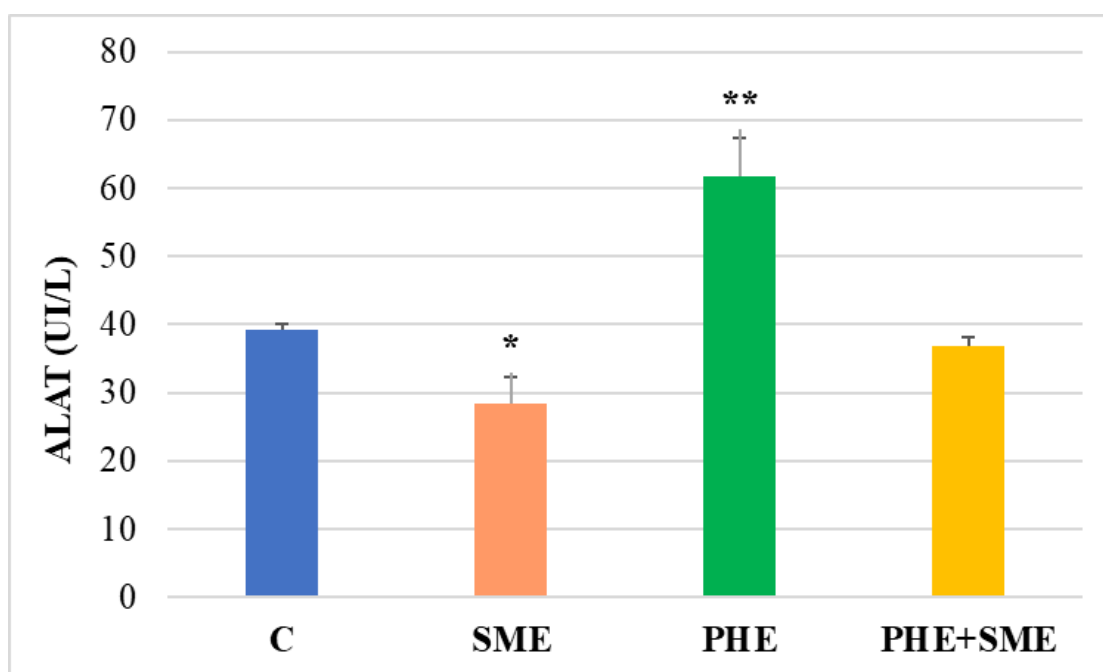


Figure 22. Serum ALAT activity (UI/L) in the different experimental groups (* $P < 0,05$ ** $P < 0,01$ relative to the control group (C)) .

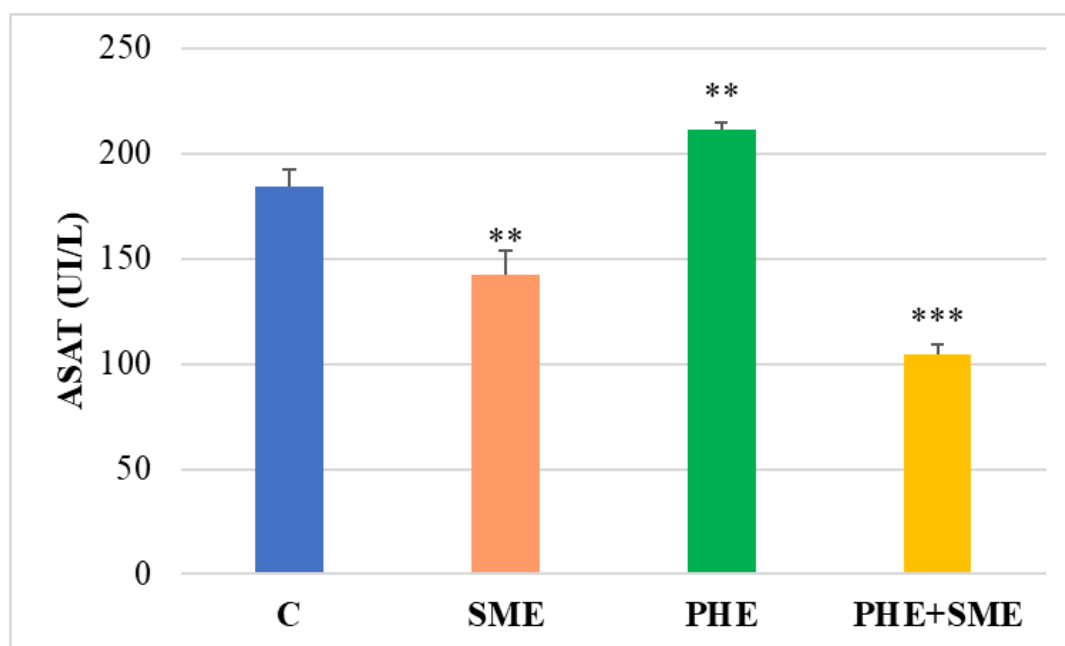


Figure 22. Serum ASAT activity (UI/L) in the different experimental groups. (* $P < 0,05$ ** $P < 0,01$ relative to the control group (C)) .

In the evaluation of hepatic integrity, aminotransferases serve as critical indicators of cellular damage. Alanine aminotransferase (ALT) is a specific cytosolic enzyme found primarily in hepatocytes, making its elevated serum levels a highly sensitive biomarker for acute liver injury (**Gowda et al., 2009**). On the other hand, aspartate aminotransferase (AST) is present in both the cytoplasm and mitochondria of metabolically active tissues, including the liver, heart, and skeletal muscles, serving as a reliable indicator of cellular necrosis and mitochondrial dysfunction when evaluated alongside ALT (**Gowda et al., 2009; Jaffar et al., 2024**).

In this study, the observed elevation in both ALT and AST activities following exposure to *Peganum harmala* extract (PHE) strongly indicates severe hepatocellular injury and intoxication, a finding that aligns with previous reports (**Wang et al., 2019**). This hepatotoxicity is primarily attributed to the plant's β -carboline alkaloids, such as harmine and harmaline. These active compounds are well-documented to induce oxidative stress, mitochondrial dysfunction, and lipid peroxidation, which ultimately impair cellular antioxidant defenses, leading to hepatocyte membrane destabilization and the subsequent leakage of these enzymes into the circulation (**Wang et al., 2019; Li, 2024**).

Conversely, the significant decrease in both ALT and AST activities observed after *Silybum marianum* extract (SME) administration firmly supports its potent hepatoprotective and therapeutic role (Dhande *et al.*, 2024). This protective mechanism is mainly driven by silymarin, a flavonolignan complex renowned for its exceptional antioxidant, anti-inflammatory, and membrane-stabilizing properties (Jaffar *et al.*, 2024). Recent literature confirms that silymarin supplementation successfully restores liver enzyme activities and enhances antioxidant defense systems during hepatic damage (El-Demerdashet *al.*, 2024; Dhande *et al.*, 2024; Jaffar *et al.*, 2024). By effectively mitigating oxidative stress, reducing lipid peroxidation, and preserving structural membrane integrity against *Peganum harmala*-induced alterations, SME prevents hepatocyte structural breakdown, limits enzyme leakage, and successfully restores these biochemical parameters toward baseline levels (El-Demerdashet *al.*, 2024; Jaffar *et al.*, 2024).

4.2.4. Effect of treatment on albumine levels

In the present study, the analysis of serum albumin levels revealed no marked or statistically significant alterations among the experimental groups compared with the control group (C). Although slight variations were observed between the treated groups, albumin concentrations remained relatively stable throughout the experiment. This stability suggests that despite the induction of hepatic alterations, the liver's protein synthesis machinery was not severely impaired under these experimental conditions.

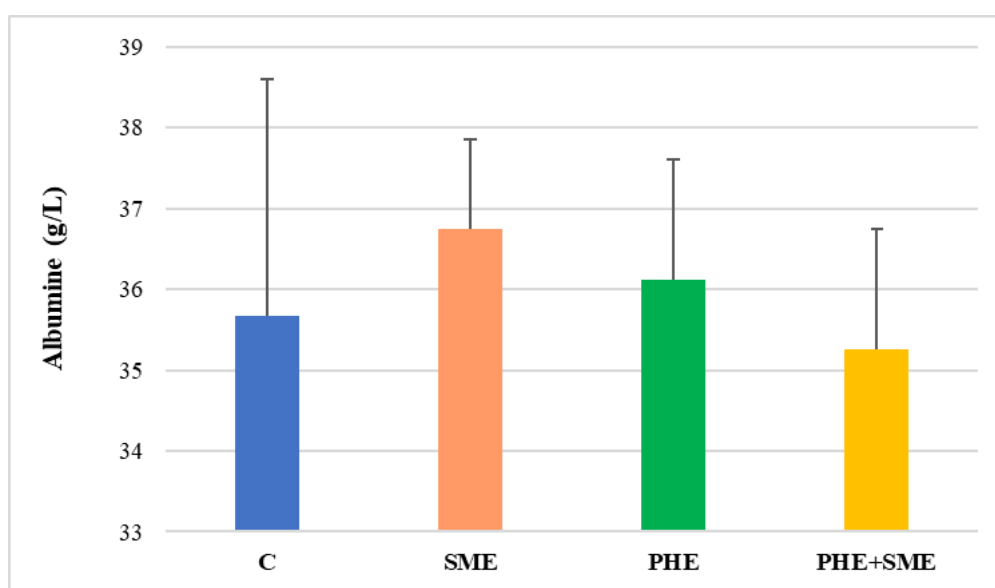


Figure 24. Serum albumin concentration (g/L) in the different experimental groups. The absence of significant changes in serum albumin suggests that the hepatotoxic effect

induced by *P. harmala* was primarily reflected in markers of hepatocellular injury (such as AST and ALT), rather than in liver synthetic capacity. This pattern is commonly observed in mild to moderate hepatic injury,

Serum albumin is the most abundant plasma protein synthesized exclusively by hepatocytes. It plays a critical role in maintaining oncotic pressure and serves as a primary indicator of hepatic synthetic capacity and functional integrity (Levitt & Levitt, 2016).

where enzyme leakage occurs due to membrane damage while protein synthesis remains relatively preserved. Similar findings have been reported in experimental studies where exposure to hepatotoxic agents led to elevated transaminases without significant changes in serum albumin, indicating that albumin is a relatively late and insensitive marker of acute liver dysfunction (Ozer *et al.*, 2008).

4.3.Oxidative Stress parameters

4.3.1.The effects of the administered plant extracts on hepatic non-protein reduced glutathione (GSH) levels

The control (C) and *Silybum marianum* extract (SME) groups exhibited stable physiological baseline levels of GSH, quantified at approximately 0.0023 and 0.0018 mmol/mg respectively. In contrast, the administration of *Peganum harmala* extract (PHE) induced a statistically significant increase ($p < 0.05$) in hepatic GSH reserves, which peaked sharply at approximately 0.0041 mol/mg. This abrupt surge represents an active, adaptive compensatory mechanism by the hepatocytes to counteract severe, acute oxidative insults. However, when *Silybum marianum* extract was co-administered alongside the toxic plant extract (PHE + SME group), it successfully mitigated the chemical stress, restoring the GSH levels back to the baseline control range (0.0023 mmol/mg) and demonstrating a clear hepatoprotective effect.

Glutathione (GSH) serves as the primary non-enzymatic intracellular antioxidant shield against circulating xenobiotics and free radicals. In this investigation, the acute exposure to *Peganum harmala* extract (PHE) triggered an immediate up-regulation of hepatic GSH synthesis. This immediate biochemical rebound is a typical cellular response to neutralize the heavy alkaloidal load present within the plant.

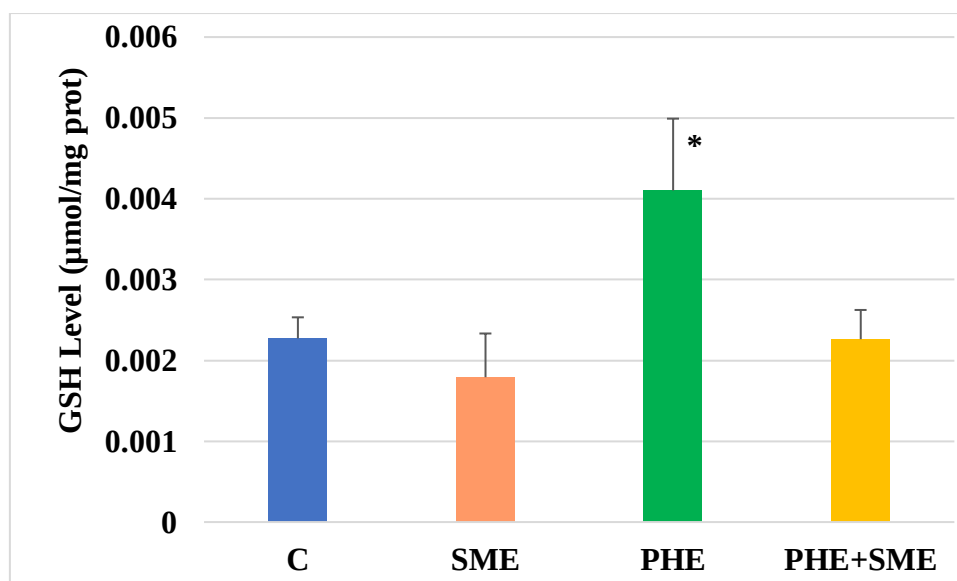


Figure 25. Bar chart representing tissue reduced glutathione (GSH) levels (mol/mg protein) in the different experimental groups (*P< 0,05 relative to the control group (C)).

Reduced Glutathione (GSH) is a linear tripeptide composed of L-glutamine, L-cysteine, and glycine that serves as the primary non-enzymatic intracellular antioxidant in eukaryotic cells (**Forman and Zhang, 2024**). It acts as a vital nucleophilic co-substrate to directly scavenge reactive oxygen species (ROS), maintain cellular thiol redox status, and neutralize toxic xenobiotics through conjugation reactions (**Forman and Zhang, 2024**).

This observation is strongly supported by **Bouaslaet al.(2024)**, who demonstrated that acute, early-stage toxicity induced by *P. harmala* alkaloids (notably harmine and harmaline) causes a temporary, defensive overproduction of endogenous GSH to shield cellular proteins from covalent binding and damage.

Upon co-administration of *Silybum marianum* extract (SME), this burden was lifted, and GSH levels normalized. This outcome is credited to silymarin, the active bioflavonoid complex in milk thistle, which directly scavenges reactive chemical intermediates. As highlighted by **Ranjan and Gautam (2023)**, the presence of silymarin prevents metabolic exhaustion in hepatocytes by absorbing the primary oxidative stress load, thereby eliminating the need for the liver to undergo a resource-heavy compensatory GSH overproduction.

4.3.2.The effects of the administered plant extracts on Catalase (CAT) enzymatic Activity

The control (C) and SME groups maintained steady, physiological baseline enzyme activities measured at roughly 81 and 85 $\mu\text{mol}/\text{min}/\text{mg}$ prot, respectively. Following the administration of *Peganum harmala* extract (PHE), a statistically significant increase ($p < 0.05$) in CAT activity was observed, accelerating past 100 $\mu\text{mol}/\text{min}/\text{mg}$. This robust enzymatic induction signals a high production rate of intracellular peroxides driven by PHE toxicity, forcing the tissue to over-express the enzyme to avoid structural cellular damage. Notably, the combination of *Silybum marianum* with the toxic extract in the PHE + SME group successfully suppressed this over-activation, adjusting catalase kinetics back to normal physiological values (74 $\mu\text{mol}/\text{min}/\text{mg}$).

Catalase (CAT) is a fundamental peroxisomal antioxidant enzyme that catalyzes the dismutation of dangerous hydrogen peroxide (H_2O_2) into harmless water and oxygen. The marked stimulation of CAT activity observed in the PHE group demonstrates a severe intracellular accumulation of reactive oxygen species (ROS) that forced the hepatocytes to accelerate enzyme expression as a survival strategy.

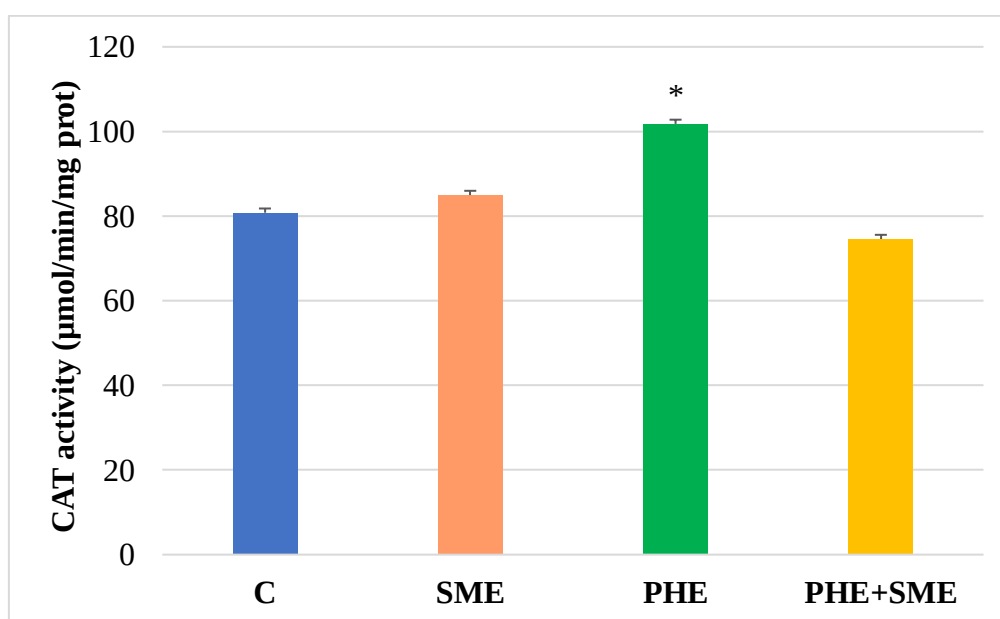


Figure 26. Bar chart representing catalase (CAT) activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein) in the different experimental groups (* $P < 0.05$ relative to the control group (C)).

Catalase is a highly conserved, tetrameric heme-containing antioxidant enzyme predominantly localized within cellular peroxisomes (**Goyal and Basak, 2023**). It catalyzes the extremely efficient dismutation of toxic hydrogen peroxide (H_2O_2) into harmless molecular oxygen (O_2) and water (H_2O), thereby preventing the intracellular accumulation

of hydroxyl radicals and safeguarding hepatocytes against lipid peroxidation (**Goyal and Basak, 2023**).

These findings correlate closely with **Khediret *al.*,(2023)**, who established that exposure to alkaloid-rich botanical toxins induces mitochondrial and peroxisomal stress, leading to massive H₂O₂ generation and an obligatory upregulation of catalase transcription to prevent downstream lipid peroxidation and DNA damage.

Conversely, the restoration of baseline CAT activity in the PHE + SME group highlights the proactive cellular shielding provided by *Silybum marianum*. Recent data published by **Abdel-Moneim *et al.*(2026)** notes that active components in milk thistle function as high-efficiency metabolic shields that directly stabilize free radicals before they can transition into complex peroxides. By minimizing upstream ROS formation, SME limits the operational stress placed on endogenous enzyme networks, allowing catalase levels to return to healthy, balanced baselines.

Conclusion and Perspectives

Conclusion and Perspectives

This experimental study represents a pivotal step toward understanding the delicate balance between the therapeutic activities and toxicological profiles of medicinal plants commonly used in traditional medicine. The biochemical and physiological results obtained over 45 days of rigorous monitoring demonstrated that the excessive administration of *Peganum harmala* extracts at uncalibrated doses (60 mg/kg) induces acute and severe structural hepatotoxicity.

This hepatic impairment is clinically manifested by a sharp, excessive elevation of liver enzymes indicative of cellular damage, such as aminotransferases (AST and ALT), along with disruptions in metabolic indicators including triglycerides and albumin. Furthermore, it triggers intense intracellular oxidative stress within hepatocytes, characterized by the depletion of endogenous antioxidant defenses and kinetic alterations of the catalase enzyme resulting from the massive accumulation of reactive oxygen species (ROS).

Conversely, this study opens a promising therapeutic avenue by demonstrating the high hepatoprotective efficacy of *Silybum marianum* extract at a dose of (80 mg/kg). The combined treatment (PHE + SME) highlighted the outstanding capacity of milk thistle to act as a metabolic shield and bio-antioxidant agent.

The extract successfully stabilized hepatic cell membranes, contributed to protecting them against oxidative damage, and restored catalase activity and biochemical markers (AST, ALT) to their normal physiological baselines. This efficacy is primarily attributed to the richness of *Silybum marianum* in silymarin, which functions as a potent scavenger of free radicals and a stimulator of self-repair mechanisms within cells against alkaloid-induced toxicity.

Based on these scientific findings, and given that scientific research is an ongoing cumulative process, we recommend expanding future perspectives beyond laboratory characterization toward a prescriptive application. This should be achieved by pursuing deeper histopathological and molecular investigations into *Peganum harmala* alkaloids, alongside the precise pharmaceutical isolation of flavonolignans from *Silybum marianum*. Consequently, this positions us before the following fundamental prescriptive question "What is the optimal strategy to legislatively restrict the unregulated consumption of *Peganum harmala* based on its toxicological profiles, while mandating the integration of the active

principles of *Silybum marianum* as a natural antidote and metabolic shield to protect hepatocytes within the national pharmaceutical system ? "

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