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Assessment of the protective effects of
anacyclus pyrethrum and *cichorium intybus*
combination on hepatotoxicity in rats

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Dedication

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ABSTRACT:

The present study aimed to evaluate the hepatoprotective effects of *Anacyclus pyrethrum* and *Cichorium intybus*, administered individually or in combination, against paracetamol-induced hepatotoxicity in rats. Previous studies have demonstrated that both plants possess important biological properties, including antioxidant, detoxifying, and anti-inflammatory activities, which may contribute to hepatic protection. To investigate the effects of *A. pyrethrum* and *C. intybus* administration on liver structure and function, histopathological evaluation, biochemical analysis, and oxidative stress assessment were performed. Forty male Wistar rats were randomly divided into eight experimental groups, including control group, 3 plant-treated groups (*A. pyrethrum*, *C. intybus* and mixture), intoxicated group, and 3 treatment (*A. pyrethrum*, *C. intybus* and mixture) + intoxication groups. Plant extracts and their mixture (50:50) were administered orally at a dose of 200 mg/kg b.w. daily for seven days. Hepatotoxicity was induced in the intoxicated and treatment-intoxicated groups by administration of paracetamol at a toxic single dose (3g/ Kg b.w) on the eighth day. At the end of the experimental period, blood and liver tissues were collected for biochemical, oxidative stress, and histopathological investigations. The results demonstrated that paracetamol intoxication induced severe hepatic injury, characterized by significant increases ($p < 0.001$) in serum transaminases (TGO and TGP), total and direct bilirubin, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), accompanied by a marked reduction ($p < 0.001$) in total protein levels compared with the control group. These biochemical disturbances were associated with oxidative stress, evidenced by reduced catalase (CAT) activity and increased malondialdehyde (MDA) levels, indicating impairment of antioxidant defenses and enhanced lipid peroxidation. Histopathological examination of intoxicated animals revealed marked hepatic alterations, including lobular lymphocytic inflammatory infiltration, hepatocellular ballooning, cytoplasmic clarification, and disruption of normal hepatic architecture. In contrast, preventive treatment with *A. pyrethrum*, *C. intybus*, and their combination significantly improved liver biochemical parameters by reducing hepatic enzyme leakage, normalizing bilirubin concentrations, restoring total protein levels, increasing CAT activity, and decreasing MDA concentrations compared with intoxicated animals. Among the tested treatments, *C. intybus* exhibited greater hepatoprotective activity than *A. pyrethrum*, while the combination of both extracts produced the strongest protective effect, suggesting possible synergistic interactions between their bioactive compounds. Histological analysis further confirmed these findings, showing near-normal hepatic architecture with only mild inflammatory infiltration and absence of major degenerative changes. Overall, the findings suggest that preventive administration of *A. pyrethrum* and *C. intybus*, particularly in combination, effectively attenuates oxidative stress, preserves hepatic architecture, and protects against paracetamol-induced hepatotoxicity.

Keywords: hepatoprotection, *Anacyclus pyrethrum*, *Cichorium intybus*, combination, paracetamol, liver.

Résumé

La présente étude avait pour objectif d'évaluer les effets hépatoprotecteurs d'*Anacyclus pyrethrum* et de *Cichorium intybus*, administrés individuellement ou en association, contre l'hépatotoxicité induite par le paracétamol chez le rat. Des études antérieures ont montré que ces deux plantes possèdent d'importantes propriétés biologiques, notamment des activités antioxydantes, anti-inflammatoires et détoxifiantes, susceptibles de contribuer à la protection hépatique. Afin d'étudier les effets d'*A. pyrethrum* et de *C. intybus* sur la structure et la fonction du foie, des analyses biochimiques, du stress oxydatif et histopathologiques ont été réalisées. Quarante rats mâles Wistar ont été répartis aléatoirement en huit groupes expérimentaux : un groupe témoin, trois groupes traités par les plantes (*A. pyrethrum*, *C. intybus* et leur mélange), un groupe intoxiqué et trois groupes prétraités puis intoxiqués. Les extraits végétaux et leur mélange (50:50) ont été administrés par voie orale à la dose de 200 mg/kg de poids corporel par jour pendant sept jours. L'hépatotoxicité a été induite le huitième jour chez les groupes intoxiqués par l'administration d'une dose toxique unique de paracétamol (3 g/kg de poids corporel). À la fin de la période expérimentale, des échantillons sanguins et hépatiques ont été prélevés pour les analyses biochimiques, du stress oxydatif et histopathologiques. Les résultats ont montré que l'intoxication au paracétamol provoquait une atteinte hépatique sévère, caractérisée par une augmentation significative ($p < 0,001$) des transaminases sériques (TGO/AST et TGP/ALT), de la bilirubine totale et directe, de la gamma-glutamyl transférase (GGT) et de la phosphatase alcaline (ALP), associée à une diminution significative ($p < 0,001$) des protéines totales par rapport au groupe témoin. Ces perturbations biochimiques étaient accompagnées d'un stress oxydatif, mis en évidence par une diminution de l'activité de la catalase (CAT) et une augmentation des taux de malondialdéhyde (MDA), traduisant une altération des défenses antioxydantes et une intensification de la peroxydation lipidique. L'examen histopathologique des animaux intoxiqués a révélé des lésions hépatiques marquées, notamment une infiltration inflammatoire lymphocytaire lobulaire, une ballonnisation des hépatocytes, une clarification cytoplasmique et une altération de l'architecture hépatique normale. En revanche, le prétraitement par *A. pyrethrum*, *C. intybus* et leur association a significativement amélioré les paramètres biochimiques hépatiques en réduisant la fuite des enzymes hépatiques, en normalisant les taux de bilirubine, en restaurant les concentrations de protéines totales, en augmentant l'activité de la CAT et en diminuant les niveaux de MDA par rapport aux animaux intoxiqués. Parmi les traitements étudiés, *C. intybus* a présenté une activité hépatoprotectrice plus importante que *A. pyrethrum*, tandis que l'association des deux extraits a produit l'effet protecteur le plus marqué, suggérant une interaction synergique entre leurs composés bioactifs. Les observations histologiques ont confirmé ces résultats en montrant une architecture hépatique presque normale, avec seulement une légère infiltration inflammatoire et l'absence de modifications dégénératives majeures. En conclusion, l'administration préventive d'*A. pyrethrum* et de *C. intybus*, particulièrement en association, atténue efficacement le stress oxydatif, préserve l'architecture hépatique et protège contre l'hépatotoxicité induite par le paracétamol chez le rat.

Mots-clés : Hépatoprotection, *Anacyclus pyrethrum*, *Cichorium intybus*, combinaison, paracétamol, atteinte hépatique.

الملخص

هدفت هذه الدراسة إلى تقييم التأثيرات الواقية للكبد النباتي *Anacyclus pyrethrum* (عافر قرحا) و (الهندباء البرية) *Cichorium intybus* ، سواء عند إعطائهما بشكل منفرد أو في صورة مزيج، ضد السمية الكبدية المستحثة بالباراسيتامول لدى الجرذان. وقد أظهرت دراسات سابقة أن هذين النباتين يمتلكان خصائص بيولوجية مهمة، من بينها النشاط المضاد للأكسدة والمضاد للالتهاب والمزيل للسموم، مما قد يساهم في حماية الكبد. بد. لدراسة تأثيرات *A. pyrethrum* و *C. intybus* على بنية ووظيفة الكبد، أُجريت تحاليل بيوكيميائية ودراسات للإجهاد التأكسدي وفحوصات نسيجية مرضية. تم تقسيم أربعين جرذاً من ذكور الويستر عشوائياً إلى ثماني مجموعات تجريبية: مجموعة شاهدة، وثلاث مجموعات معالجة بالنباتات *A. pyrethrum* ، *C. intybus* ، والمزيج بينهما)، ومجموعة مصابة بالتسمم، وثلاث مجموعات معالجة مسبقاً بالنباتات ثم مُعرضة للتسمم. أُعطيت المستخلصات النباتية ومزيجها بنسبة (50:50) عن طريق الفم بجرعة 200 ملغ/كغ من وزن الجسم يومياً لمدة سبعة أيام. وفي اليوم الثامن، تم إحداث السمية الكبدية في المجموعات المخصصة لذلك من خلال إعطاء جرعة سامة مفردة من الباراسيتامول مقدارها 3 غ/كغ من وزن الجسم. في نهاية الفترة التجريبية، جُمعت عينات الدم وأنسجة الكبد لإجراء التحاليل البيوكيميائية ودراسات الإجهاد التأكسدي والفحص النسيجي المرضي. أظهرت النتائج أن التسمم بالباراسيتامول تسبب في إصابة كبدية شديدة، تجلت بارتفاع معنوي ($p < 0.001$) في مستويات إنزيمات الكبد (TGO) و (TGP)، والبيليروبين الكلي والمباشر، وإنزيم غاما غلوتاميل ترانسفيراز (GGT)، والفوسفاتاز القلوية (ALP)، إضافة إلى انخفاض معنوي ($p < 0.001$) في مستوى البروتينات الكلية مقارنة بالمجموعة الشاهدة. كما ارتبطت هذه التغيرات بحدوث إجهاد تأكسدي تمثل في انخفاض نشاط إنزيم الكاتالاز (CAT) وارتفاع مستويات المالوندايالديهايد (MDA)، مما يدل على تراجع الدفاعات المضادة للأكسدة وزيادة بيروكسدة الدهون. وكشف الفحص النسيجي المرضي للحيوانات المتسمة عن تغيرات كبدية واضحة شملت ارتشاحاً التهابياً لمفاوياً على مستوى الفصيصات الكبدية، وانتفاخ الخلايا الكبدية، وتصفية السيتوبلازم، واضطراب البنية الطبيعية للكبد. في المقابل، أدى العلاج الوقائي بـ *A. pyrethrum* و *C. intybus* ومزيجهما إلى تحسين ملحوظ في المؤشرات البيوكيميائية للكبد، من خلال تقليل تسرب الإنزيمات الكبدية، وتطبيع مستويات البيليروبين، واستعادة تركيز البروتينات الكلية، وزيادة نشاط الكاتالاز، وخفض مستويات المالوندايالديهايد مقارنة بالحيوانات المتسمة. ومن بين العلاجات المدروسة، أظهر *C. intybus* فعالية أكبر في حماية الكبد مقارنة بـ *A. pyrethrum*، بينما حقق المزيج بين النباتين أقوى تأثير وقائي، مما يشير إلى وجود تأثير تآزري محتمل بين المركبات النشطة بيولوجياً الموجودة فيهما. كما أكدت النتائج النسيجية هذه الملاحظات، حيث أظهرت بنية كبدية شبه طبيعية مع ارتشاح التهابي خفيف فقط وغياب التغيرات التنكسية الشديدة. بشكل عام، تشير النتائج إلى أن الإعطاء الوقائي لـ *A. pyrethrum* و *C. intybus*، وخاصة عند استخدامهما معاً، يساهم بفعالية في التخفيف من الإجهاد التأكسدي، والحفاظ على البنية النسيجية للكبد، والحماية من السمية الكبدية الناتجة عن الباراسيتامول لدى الجرذان.

الكلمات المفتاحية: الحماية الكبدية، عافر قرحا (*Anacyclus pyrethrum*) ، الهندباء البرية (*Cichorium intybus*) ، المزيج النباتي، الباراسيتامول، إصابة الكبد.

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Abbreviations list:

ALD: alcohol-associated liver disease
ALT/TGP: alanine aminotransferase
ALP: alkaline phosphatase
ANOVA: analysis of variance
AP: anacyclus pyrethrum
ARE: antioxidant response element
AREs: antioxidant response elements
AST/TGO: aspartate aminotransferase
BHT: butylhydroxytoluene
BSA: bovine serum albumin
BW: body weight
CAT : catalase
CI: cichorium intybus
CoQ10 : coenzyme Q10
DA: dopamine
DAMPs: damage-associated molecular patterns
DILI: drug-induced liver injury
DMSO: dimethyl sulfoxide
DNA: deoxyribonucleic acid
DNPH: dinitrophenylhydrazine
DPPH: 2,2-diphenyl-1-picrylhydrazyl
FRAP: Ferric reducing ability of plasma
GPX: glutathione Peroxidase
GRX: glutaredoxin
GGT: gamma-glutamyl transferase
GR: glutathione reductase
GSH: reduced glutathione
HAV, HEV: viral hepatitis
HBV: hepatitis B virus
HCC: hepatocellular carcinoma
HCV: hepatitis C virus
HPA: hypothalamic–pituitary–adrenal
HOCl: hypochlorous acid

HSCs: hepatic stellate cells
HSV: herpes simplex virus
H₂O₂: hydrogen peroxide
INR: international normalized ratio
KCs: kupffer cells
LSECs: liver sinusoidal endothelial cells
M: mean
MASLD: metabolic dysfunction-associated steatotic liver disease
MDA: malondialdehyde
NADPH: nicotinamide adenine dinucleotide phosphate
NAPQI: n-acetyl-p-benzoquinone imine
NO•: nitric oxide
NRF2: nuclear factor erythroid 2-related factor 2
NSAIDs: nonsteroidal anti-inflammatory drugs
OH•: hydroxyl radicals
ONOO⁻: peroxynitrite
PBS: phosphate buffer
pNPP: p-nitrophenylphosphate
p.o: per os
PRX: peroxiredoxin
PT: prothrombin time
RNS: reactive nitrogen species
ROS: reactive oxygen species
RUQ: right upper quadrant
SASP: senescence-associated secretory phenotype
SEM: standard error of the mean
SOD: superoxide Dismutase
TBA: thiobarbituric acid
TBARS complex: thiobarbituric acid reactive substances
TBS: tris buffered saline
TCA: trichloroacetic acid
TRX: thioredoxin
T3: triiodothyronine

T4 : thyroxine
5-HT: serotonin

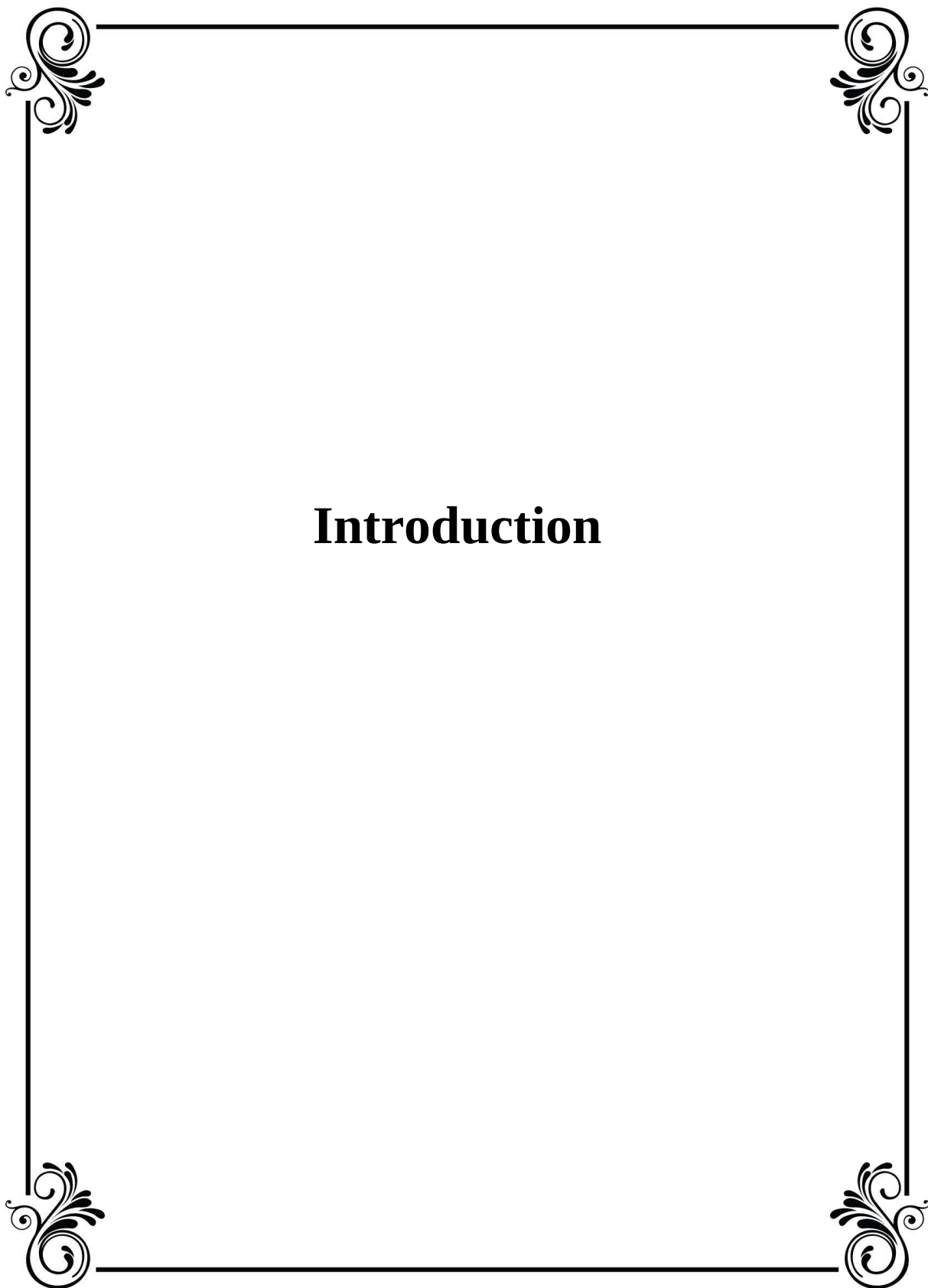
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Introduction

Introduction :

The liver is the largest internal organ in the human body and performs more than 500 essential physiological functions, including metabolism, detoxification, bile production, and protein synthesis. Owing to its central role in the biotransformation of xenobiotics and drugs, the liver is particularly susceptible to toxic injury induced by chemical agents and pharmaceuticals (Alhusaini et al., 2022).

Drug-induced liver injury represents a major health problem worldwide and is one of the leading causes of acute liver failure. Among the drugs associated with hepatotoxicity, paracetamol (acetaminophen) is widely used as an analgesic and antipyretic agent. Although safe at therapeutic doses, overdose can induce severe hepatic damage through the formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI), which depletes glutathione reserves, increases oxidative stress, and causes hepatocellular necrosis (Feki et al., 2022; Henneh et al., 2022).

Oxidative stress plays a crucial role in the pathogenesis of paracetamol-induced liver injury. Excessive generation of reactive oxygen species (ROS) disrupts the antioxidant defense system, leading to lipid peroxidation, protein oxidation, DNA damage, and ultimately liver dysfunction (Teixeira et al., 2025).

In recent years, medicinal plants have attracted considerable attention as potential sources of hepatoprotective compounds due to their richness in bioactive phytochemicals, including phenolic compounds, flavonoids, alkaloids, and terpenoids. These natural compounds exhibit antioxidant, anti-inflammatory, and cytoprotective properties that may contribute to the prevention of liver damage (Elgengaihi et al., 2016).

Among these medicinal plants, *Anacyclus pyrethrum* L. (African Pyrethrum), a member of the Asteraceae family, has been traditionally used for various therapeutic purposes. Previous studies have reported antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective activities associated with its rich phytochemical composition (Usmani et al., 2016; Elazzouzi et al., 2019).

Similarly, *Cichorium intybus* L. (chicory) is recognized as an important medicinal plant possessing antioxidant, anti-inflammatory, and hepatoprotective properties attributed to its high content of phenolic acids, flavonoids, coumarins, and other bioactive compounds (Khalid et al., 2018; Riaz et al., 2024).

Therefore, the present study was undertaken to evaluate the protective effects of *Anacyclus pyrethrum* and *Cichorium intybus*, administered individually or in combination,

against paracetamol-induced hepatotoxicity in Wistar rats, in order to assess their hepatoprotective potential and determine whether the combination of both plants could exert a synergistic protective effect. This study is divided into two principal sections: a theoretical section and an experimental section. The theoretical section addresses hepatotoxicity and reviews the biological and pharmacological characteristics of *Anacyclus pyrethrum* and *Cichorium intybus*. The experimental section includes materials and methods, followed by results and discussion, covering experimental animal design, biochemical analyses, oxidative stress assessment, and histopathological evaluation to investigate the hepatoprotective potential of the tested extracts.

Section N:01
theoretical part

Chapter I:
*Anacyclus pyrethrum &
Cichorium intybus*

1. Medicinal plants

1.1 Definition

Medicinal plants constitute one of the oldest forms of therapy, having been used for thousands of years in traditional medical practices across the world. Knowledge of their therapeutic benefits has been passed down through generations within human societies. Natural products continue to play a crucial role in drug discovery, with many modern pharmaceuticals originating from traditional herbal medicine. The biological activities of numerous plant species and their bioactive compounds have been extensively studied. Both conventional approaches, such as bioassay-guided isolation, and modern techniques including high-throughput screening and reverse pharmacognosy have facilitated the identification of a wide range of bioactive phytochemicals. Despite these advances, medicinal plants still hold significant potential, as the phytochemical composition and therapeutic properties of many species remain insufficiently explored and require further investigation (Marrelli, 2021).

1.2 Medicinal Plants in Algeria

Algeria is the largest country in the Mediterranean basin, Africa, and the Arab region with a total area of almost 2.4 million km² and 1,600 of coastline. In addition to a diversified climate, Algeria is characterized by a rich flora consisting of 4,000 taxa, 917 genera, and 131 families. Moreover, owing to its ancient history as one of the first cradles of *Homo sapiens* and civilization in the world, Algeria possesses an important and rich cultural diversity (Belhouala & Benarba, 2021).

According to a multiregional ethnobotanical study reported by Belhouala & Benarba, (2021), A total of 167 species belonging to 70 families were recorded. Lamiaceae (13%), Asteraceae (13%), Apiaceae (7%), and Rosaceae and Fabaceae (5% each) were the most cited families. The survey revealed that leaves were the most used parts of the plants (29%). Furthermore, decoction (35%), raw (24%), and infusion (19%) were the common modes for the remedies' preparation. Here, 15% of the total species were newly reported as medicinal plants. Besides, it was reported for the first time a total of 47 new therapeutic uses for 20 known plant species. Of 17 ailments categories, cancer was presented by 44 species, showing the highest informant consensus factor of 0.46. *Marrubium vulgare* L., *Artemisia herba-alba* Asso., *Zingiber officinale* Roscoe., and *Juniperus phoenicea* L. recorded the maximum fidelity value of 100%.

1.3 Extraction Methods

Extraction, as the term uses pharmaceutically, involves the separation of medicinally active compounds of plant or animal tissues from the inactive or inert components (desired and undesired) by using selective solvents in standard extraction procedures. The products obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing. The conventional extraction methods, including Maceration, Percolation and Soxhlet extraction, usually use organic solvents and require a large volume of solvents and long extraction time whereas decoction and Hydro distillation methods use water as a solvent (**Rasul, 2018**). The common conventional extraction methods used have been discussed below

Table 1: Solvents used for active component extraction (**Rasul, 2018**).

Water	Ethanol	Methanol	Chloroform	Dichlorome thanol	Ether	Aceto ne
Tannins Anthocyanin Terpenoids Saponins	Tannins Terpenoids polyphenol Flavonoids Alkaloids	Tannins Terpenoids polyphenol Saponins Anthocyanin	Flavonoids Terpenoids	Terpenoids	Alkaloids Terpenoids	Flavo noids

1.4 Bioactive Compounds

Bioactive compounds are defined as biologically reacting substances that stimulate a response in living tissue, indicating their biological activity. These compounds can be extracted and purified for various biotechnological and pharmaceutical applications and are recognized for their potential benefits in enhancing human well-being (**Dash et al., 2022**). The plant kingdom represents a vast reservoir of biologically active molecules, including terpenes, polyphenols, carotenoids, and saponins, each exhibiting diverse therapeutic properties, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities (**El-Saadony et al., 2025**).

2. *Anacyclus pyrethrum*

2.1 Definition

Anacyclus pyrethrum (L), commonly known as African pyrethrum, akarkarha, tigendesste, or igendess, is a species of the Asteraceae family native to Morocco, Algeria, and Spain. This species comprises two recognized varieties: *Anacyclus pyrethrum* var. *pyrethrum* (L) and *Anacyclus pyrethrum* var. *depressus* (Ball) Maire. In traditional medicine, the roots of *A. pyrethrum* are widely used for a range of ailments, including toothaches, increased salivary secretion, angina, digestive disorders, lethargy, female infertility, and paralysis of the tongue or limbs. They are also applied in the form of creams based on animal fats to treat gout, sciatica, and to help prevent various illnesses (Jawhari et al., 2020).

2.2 Morphology

Anacyclus pyrethrum is a perennial herb of the Asteraceae family, typically growing 40–60 cm tall. It has numerous hairy or pubescent stems arising from the base, bearing finely divided, feathery, or oblong basal leaves arranged in a rosette. The plant produces daisy-like flowers with white ray florets (purple on the underside) and yellow central discs, blooming between May and August. Its roots are long, thick, fibrous, aromatic, brown externally and white internally, often tapering or obliquely conical, with few branches. The involucre bracts are oblong, obtuse, sparsely puberulent, and lilac or grayish-white, while the fruit is an achene, either smooth or slightly crowned (El Mokhtari et al., 2020; Thakur et al., 2023; Tuersong et al., 2024).



Figure 1: The morphological appearance of *A. Pyrethrum* (Tuersong et al., 2024).

2.3 Taxonomy and the Geographical Distribution

Anacyclus pyrethrum, commonly known as African pyrethrum or “Oud El Attas,” is a medicinal plant native to the Mediterranean region, particularly Morocco, Algeria, and Spain. It is also found across Southern Europe, parts of Arabia, Syria, Xinjiang (China), and regions of India such as Jammu and Kashmir and Bengal. In Algeria, it grows wild in arid, rocky, and mountainous areas, including high-altitude regions like the Atlas Mountains, in English named Pellitory (**Usmani et al., 2016; Baslam et al., 2024**). Arabe and local names include Awd al-Attas, Akkar Karra, Tighuendeste, Ighuendes Hallala, Arq-Echlouh, Aqirqarha, and Kûkû (**Nassiri, 2020**).

Table 2: Taxonomy of *Anacyclus pyrethrum* (**Usmani et al., 2016**).

Kingdom	Plantae
Division	Spermatophyta
Sub-division	Angiosperms,
Class	Dicotyledons,
Sub class	Metachlamydae
Order	Companulatae
Family	Asteraceae
Genus	<i>Anacyclus</i>
Species	<i>Pyrethrum</i>

2.4 Chemical composition

Anacyclus pyrethrum contains a wide range of bioactive compounds distributed across its different plant parts. Phytochemical analyses of roots, leaves, and flowers reveal the presence of alkaloids, tannins, flavonoids, triterpenes, sterols, coumarins, glycosides, sugars, mucilage, and essential oils, along with trace elements such as Zn, Fe, Cu, and others. The flowers are particularly rich in flavonoids and total phenolic compounds, whereas the roots are characterized by a high content of alkaloids and resinous substances. The aerial parts mainly contain tannins and flavonoids. Notable compounds identified in the roots include pellitorine, anacyclin, phenylethylamine, inulin, sesamin, and several polyacetylenic amides (alkamides), which are responsible for many of the plant’s biological activities, including its traditional aphrodisiac properties (**Pandey et al., 2018**). *Anacyclus pyrethrum* contains a diverse range of chemical constituents, including both volatile and non-volatile compounds (**Table 4**).

Table 3 : Chemical Composition of *A.pyrethrum* (Tuersong et al., 2024).

Molecular Name	Molecular Formula	Molecular Name	Molecular Formula
Pellitorine	C ₁₄ H ₂₅ NO	-(2- phenylethyl)undeca-2,4-dien-8,10-diynamide	C ₁₉ H ₁₉ NO
Anacyclin	C ₁₈ H ₂₅ NO	(2E,4E)-dodeca-2,4- dienoic acid	C ₁₂ H ₂₀ O ₂
Dehydromatricaria ester	C ₁₁ H ₈ O ₂	(2E,4E)-N-(2-methylpropyl)dodeca- 2,4-dien-8,10-diynamide	C ₁₆ H ₂₁ NO
2,4-Dodecadienamide, N-(2-methylpropyl) -, (2E,4E) -	C ₁₆ H ₂₉ NO	N-[2-(4- hydroxyphenyl)ethyl] deca- 2,4-dienamide	C ₁₈ H ₂₅ NO ₂
N-Isobutyl-(2E,4E)-tetradecadienamide	C ₁₈ H ₃₃ NO	N-(2- Methylpropyl)dodeca- 2,4-diene-8,10- diynamide	C ₁₆ H ₂₁ NO
(2E,4E)-N-(4-Hydroxyphenethyl)-2,4-decadienamide	C ₁₈ H ₂₅ NO ₂	(2E,4E)-N-[2-(4-hydroxyphenyl)ethyl]dodeca- 2,4-dienamide	C ₂₀ H ₂₉ NO ₂
(2E,4E)-N- phenethylundeca-2,4-dien-8,10-diynamide	C ₁₉ H ₁₉ NO	N-(4- Hydroxyphenethyl)-2,4-dodecadienamid	C ₂₀ H ₂₉ NO ₂
(2E,4E,12E)-N-Isobutyl-2,4,12-tetradecatriene- 8,10-diynamide	C ₁₈ H ₂₃ NO	Sesamin	C ₂₀ H ₁₈ O ₆
N-(2- methylpropyl)dec-2-en-4,6,8-triynamide	C ₁₄ H ₁₅ NO	Dodeca-2,4-dienoic acid	C ₁₂ H ₂₀ O ₂
N-methyl-N-(2-methylpropyl)dec-2-en- 4,6-diynamide	C ₁₅ H ₂₁ NO	N-(4- Hydroxyphenethyl)-2,4-tetradecadienamid	C ₂₂ H ₃₃ NO ₂
Tetradec-6-en-8,10,12- triyn-3-one	C ₁₄ H ₁₄ O	Tannin	C ₄₂ H ₃₂ O ₂₆
Methyl 2-decene-4,6,8-triynoate	C ₁₁ H ₈ O ₂	Coumarin	C ₉ H ₆ O ₂
Trideca-3,5-dien-7,9,11- triyn-1-ol	C ₁₃ H ₁₂ O	Sterol	C ₁₇ H ₂₈ O
N-methyl-N-(2-methylpropyl)deca- 2,8-dien-4,6-diynamide	C ₁₅ H ₁₉ NO	Spathulenol	C ₁₅ H ₂₄ O
(3E,5E)-trideca-3,5-dien-7,9,11-triyn-1-ol	C ₁₃ H ₁₂ O	Agrocybenine	C ₁₂ H ₁₈ N ₂ O
2-Decene-4,6,8-triynoic acid, methyl ester, (Z)-	C ₁₁ H ₈ O ₂		

Its volatile oil fraction comprises compounds such as spathulenol, salvial-1-one, and caryophyllene derivatives, whose concentrations may vary depending on the plant's growth stage, thereby influencing its aroma and biological activity. The non-volatile fraction includes alkaloids, phenolic compounds, and terpenoids; notably, alkaloids like pellitorine exhibit anti-inflammatory

and analgesic effects, while phenolic compounds (e.g., flavonoids) contribute antioxidant and anti-inflammatory properties. Terpenoids, particularly alkylamides, are associated with multiple biological activities, including antimicrobial, antioxidant, and immunomodulatory effects. Additionally, specific compounds such as pyrethrins and anacyclins have been identified, known for their insecticidal and anti-inflammatory activities. Overall, the complex and heterogeneous chemical composition of *A. pyrethrum* underlies its broad pharmacological potential; however, further studies are needed to better understand the relationship between its constituents and biological effects, as well as to evaluate its safety and possible toxicity for clinical use (Tuersong et al., 2024).

2.5 Pharmacological activity

2.5.1 Antioxidant activity

Anacyclus pyrethrum has been widely studied for its antioxidant properties, with various extracts showing significant activity depending on the solvent, plant part, and origin. Methanolic extracts generally exhibit the strongest antioxidant capacity, followed by aqueous and other solvent fractions, as demonstrated by assays such as DPPH and FRAP. Roots, leaves, flowers, and seeds all contribute to antioxidant activity, with particularly high potency observed in the leaves of *A. pyrethrum* var. *depressus* and the roots of var. *pyrethrum*. This activity is mainly attributed to the presence of phenolic compounds, flavonoids, and alkylamides. Overall, the antioxidant potential of *A. pyrethrum* varies across studies but consistently highlights the plant as a rich source of natural antioxidants (Selles et al., 2012; Elazzouzi et al., 2019).

2.5.2 Antimicrobial activity

Phenolic and flavonoid compounds are major constituents of *Anacyclus pyrethrum* and are responsible for its diverse biological activities, particularly antioxidant and antimicrobial effects. Among different extracts, the methanolic extract shows the strongest antimicrobial activity against a wide range of microorganisms, including *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, and *Candida albicans*. Overall, *A. pyrethrum* has demonstrated activity against several bacterial genera such as *Bacillus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Salmonella*, and *Staphylococcus*, as well as fungi like *Candida*, although some strains, including *Enterococcus faecalis* and *Salmonella typhimurium*, show resistance. Antimicrobial effectiveness varies depending on the extract type and concentration, with root ethanolic extracts generally exhibiting the highest inhibitory activity compared to leaves and stems (Selles et al., 2012; Canli et al., 2017).

2.5.3 Antidiabetic effect

Since ancient times, medicinal plants have been widely used as complementary therapies for managing diabetes and its complications. Among these, *Anacyclus pyrethrum* has been recognized in traditional medicine for its antidiabetic potential. Experimental studies have demonstrated that root extracts of this plant can significantly improve diabetic conditions. In vivo investigations on diabetic rats have shown that both alcoholic and aqueous root extracts reduce elevated blood glucose levels and restore them close to normal values. In addition, in vitro studies reveal that the ethanolic root extract exhibits strong α -amylase inhibitory activity, indicating its ability to regulate carbohydrate metabolism. These effects are mainly attributed to bioactive compounds such as alkylamides, alkaloids, and phenolic constituents. Overall, *A. pyrethrum* shows promising antidiabetic properties; however, further in vivo and clinical studies are necessary to confirm its efficacy and safety for therapeutic use (Elazzouzi et al., 2022).

2.5.4 Neurological effects

Anacyclus pyrethrum extract has shown significant antidepressant effects in various animal models. Its bioactive constituents, including fatty acids, N-alkylamides, and alkaloids, help alleviate depressive symptoms by regulating the hypothalamic–pituitary–adrenal (HPA) axis and reducing corticosterone levels. Experimental studies have demonstrated that the extract improves behavioral parameters in depressed animals, such as increasing activity and sucrose preference while reducing immobility in forced swim and tail suspension tests. These effects are also associated with enhanced neurotransmitter activity, particularly increased levels of serotonin (5-HT) and dopamine (DA) in the brain. Additionally, the extract exhibits anxiolytic and sleep-enhancing properties, supporting its traditional use in managing mood disorders and insomnia (Tuersong et al., 2024).

The *Anacyclus pyrethrum* has shown significant memory-enhancing effects in experimental models. The central cholinergic system plays a crucial role in regulating cognitive functions, and its impairment is a key feature of Alzheimer's disease. In this condition, the loss of cholinergic neurons in the cortex contributes to memory decline. Scopolamine is commonly used to induce memory impairment, mimicking cognitive deficits. Enhancing cholinergic neurotransmission has been shown to improve memory and cognitive function (Usmani et al., 2016).

2.5.5 Aphrodisiac activity

Throughout history, humans have sought ways to enhance, maintain, or stimulate sexual function and desire, often turning to aromatic and medicinal plants. Among these, *Anacyclus pyrethrum* has been traditionally valued for its aphrodisiac properties. Research has shown that extracts from its roots can increase body and reproductive organ weight, boost sperm count, and reduce abnormal sperm in male rats. Additionally, these extracts demonstrate androgenic activity, promoting spermatogenesis and improving male fertility. They also elevate seminal fructose levels and enhance sexual behavior, with effects increasing in a dose-dependent manner (Elazzouzi et al., 2022).

2.5.6 Other activities

The aqueous-alcoholic extract of *Anacyclus pyrethrum* roots has shown a hepatoprotective effect against liver damage induced by drugs like isoniazid and rifampicin in rats. Additionally, *A. pyrethrum* exhibits anticancer potential, significantly inhibiting the growth of human colorectal carcinoma cells and inducing apoptosis (Elazzouzi et al., 2022).

The ethanolic extract of *A. pyrethrum* also demonstrates neuropharmacological activity. Its root extracts display notable immunomodulatory effects: the aqueous extract is highly active when administered orally at low doses, the methanolic extract stimulates the immune system at moderate doses, and the petroleum extract can counteract immunosuppression caused by cyclophosphamide. Overall, *A. pyrethrum* shows promising hepatoprotective, anticancer, neuropharmacological, and immunomodulatory properties (Elazzouzi et al., 2022).

3. *Cichorium intybus*

3.1 Definition

Cichorium intybus L., a member of the Asteraceae family, is an important medicinal herb widely used in traditional systems such as Ayurveda, Unani, and Siddha, particularly for the treatment of hepatobiliary and renal disorders. Phytochemical studies have identified several key constituents in chicory, including caffeic acid derivatives, flavonoids, inulin, fructooligosaccharides, and other polyphenols (Zaman & Basar, 2013).

Historically, *Cichorium intybus* has been known for over 2,500 years (Aisa et al., 2020), with its earliest use traced back to ancient Egypt. It has a long tradition of medicinal application across various civilizations, including ancient Rome, Greece, and Egypt, where it was used to enhance metabolism and aid digestion. The plant was also consumed as a vegetable and used as animal

forage. Renowned scholars such as Theophrastus, Dioscorides, Pliny the Elder, and Avicenna documented its therapeutic properties, particularly for digestive and visual disorders. Avicenna also recommended chicory root decoctions for relieving gout pain. In addition, *chicory* has been extensively used in Unani medicine and, during the medieval period in Europe, it was employed to treat conditions such as jaundice and malaria (**Janda et al., 2021**).

3.2 Morphology

Cichorium intybus L. is an annual, biennial, or perennial herb belonging to the Asteraceae family. It is a winter-hardy plant that typically grows between 20 and 150 cm in height and develops a long, thick, brown taproot. The stems are erect, rigid, and often ribbed, with few leaves and occasional reddish tinges. The leaves are green and arranged in a basal rosette measuring 10–25 cm in length. They are generally oblong, lanceolate, and may be pinnately lobed or serrated. Lower leaves are broader with distinct lobes, while upper stem leaves are narrower, less divided, and may have smooth or slightly toothed edges. Leaf surfaces may be hairy, particularly along the veins. The plant produces inflorescences composed of numerous flower heads (2–4 cm in diameter), borne singly or in small clusters on short peduncles. The flowers are typically light blue (**Figure 2**), with darker central florets, and they open mainly under sunny conditions. They are rich in nectar and pollen. The fruit is a small, light brown achene, about 2–3 mm long, with a short pappus. *Cichorium intybus* grows both wild and cultivated in temperate regions, commonly found in meadows, grasslands, wastelands, roadsides, and low mountainous areas (**Javed et al., 2020; Al-Snafi, 2016**).



Figure 2: *Cichorium intybus* L (Paniagua-Zambrana et al., 2025)

3.3 Taxonomy and geographic distribution

Cichorium intybus is commonly known as chicory, it is a perennial herb that commonly grows as a weed in clay-rich soils of grasslands and cultivated fields. It is widely distributed as a roadside wild plant across its native regions, including Western Asia, North Africa, and Europe. During early European colonization, it was introduced to North America and has since spread globally, now occurring in countries such as China and Australia (Azad et al., 2025). There are two subspecies of the genus *Cichorium* are present in Algeria, locally known as “Seriss” and “Tilfaf”: *Cichorium intybus* subsp. *intybus* and *Cichorium intybus* subsp. *pumilum*. The latter is distinguished by its smaller size and slenderer stem compared to the former (Nouasri et al., 2022).

Table 4 : *Cichorium intybus* Classification (Das et al., 2016).

Kingdom	Plantae
Subkingdom	Tracheoblonta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Tribe	Cichorieae
Genus	<i>Cichorium</i>
Species	<i>intybus</i>

The plant is active during warm seasons and becomes dormant during colder periods. Its life cycle includes four main stages: primary dormancy (seed stage), vegetative growth (seedling to mature non-reproductive plant), generative phase (reproductive stage from young to mature plants), and senescence. Rapid elongation of reproductive stems contributes to its accelerated growth, particularly in late spring and early summer (Azad et al., 2025).

3.4 Chemical composition

Cichorium intybus is rich in bioactive compounds, mainly phenolic acids (especially chlorogenic acids) and flavonoids, often present as glycosides. Its roots are particularly high in inulin, along with sugars, proteins, and minerals, while also containing sesquiterpene lactones responsible for bitterness (Table 5). The plant also includes various secondary metabolites such as alkaloids, tannins, saponins, coumarins, and volatile compounds. Its seeds are nutritionally valuable, providing proteins, essential amino acids, fatty acids, and important minerals.

Additionally, the aerial parts contain compounds like chicoric acid, flavonoids, vitamins, and anthocyanins, which contribute to its biological and nutritional properties (Nwafor et al., 2017).

Table 5 : Chemical composition of *Cichorium intybus* (Janda et al., 2021).

Part Plant	Chemical Compounds
Leaves	fatty acids: C14:0; C15:0; C16:0; C18:0; C18:1n-9c; C18:2n-6c; C18:3n-3; C20:0; C22:0; C24:0
	pigments: lutein, violaxanthin antheraxanthin, neoxanthin, chlorophyll a, chlorophyll b, pheophytin a, pheophytin b, β -carotene
	polyphenols, flavonoids, chlorogenic acid, caffeic acid, chicoric acid, quercetin glucuronide, gallic acid
	tannins, saponins
	minerals: Ca, Mg, Na, Cu, Zn, Mn, Se, N, P, K, S, B, Fe
	vitamins: A, E, K, C, B1, B2, B3, B5, B6, B9
Roots	polyphenols, flavonoids, caffeoylquinic acid,
	minerals: Ca, Mg, Na, Cu, Zn, Mn, Fe, K,
	phenolic compound: protocatechuic acid, chlorogenic acid, hydroxybenzoic acid, isovanillic acid, coumaric acid, protocatechuic acid, chlorogenic acid, caffeic acid, coumaric acid, <i>p</i> -coumaric acid
	fatty acids: C ₁₄ H ₂₈ O ₂ , C ₁₆ H ₃₀ O ₂ , C ₁₆ H ₃₂ O ₂ , C ₂₀ H ₃₄ O ₂ , C ₂₀ H ₄₀ O ₂
	steroids, terpenoids,
	vitamin C
	tannins
Seeds	amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, serine, glutamic acid, glycine, alanine, aspartic acid, proline
	fatty acids: C14:0, C16:0, C16:1, C18:0 t11-C18:1, C18:1n-9, C18:2n-6, C18:3n-3, C20:0, C20:1, C20:2, C22:0, C24:0
	minerals: P, K, Ca, Mg, Na, Fe, Cu, Zn, Mn, Mo, Se, Cd,
	steroids, terpenoids,
	vitamin C
	tannins
Flowers	fatty acids: fatty acids: C ₁₄ H ₂₈ O ₂ , C ₁₅ H ₃₀ O ₂ , C ₁₆ H ₃₂ O ₂ , C ₁₇ H ₃₄ O ₂ , C ₁₈ H ₃₂ O ₂ , C ₁₈ H ₃₆ O ₂ , C ₁₉ H ₃₈ O ₂ , C ₁₈ H ₃₀ O ₂ , C ₂₀ H ₄₀ O ₂ , C ₂₀ H ₄₀
	steroids, terpenoids,
Aerial parts	phenolic compounds: caftaric acid, chlorogenic acid, cichoric acid, isoquercitrin, rutin, quercitrin, luteolin, apigenin
	minerals: Fe, Cu, Zn, Mn
	vitamin C
	tannins

3.5 Pharmacological Activities

3.5.1 Antimicrobial Activity

Extracts of *Cichorium intybus* exhibit notable antiviral activity, particularly against herpes simplex virus (HSV-1), with moderate effects on adenovirus. Compounds such as cichoric acid have demonstrated anti-hepatitis B activity and may serve as promising candidates for antiviral drug development. Additionally, computational studies have identified *C. intybus* as a potential source of bioactive compounds effective against COVID-19, although further experimental validation is required. The plant also shows broad-spectrum antimicrobial activity against various Gram-positive and Gram-negative bacteria, as well as fungi. Different extracts (aqueous, methanolic, ethanolic, and ethyl acetate) have demonstrated inhibitory effects against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. In some cases, these extracts exhibited comparable or even stronger activity than standard antibiotics, highlighting the significant antibacterial and antifungal potential of *C. intybus* (Janda et al., 2021).

3.5.2 Antioxidant activity

The phenolic composition and antioxidant activity of *Cichorium intybus* vary among different varieties, with red types showing higher activity due to anthocyanins, particularly cyanidin derivatives. All varieties contain significant amounts of hydroxycinnamic and hydroxybenzoic acids, contributing to strong free radical scavenging capacity. Leaf extracts exhibit notable antioxidant effects, including inhibition of lipid peroxidation and reduction of oxidative stress markers. Anthocyanins are the main contributors to this activity, especially red chicory. Storage and processing have minimal impact on phenolic content, with most compounds remaining stable under refrigeration. Environmental and processing conditions, such as temperature and drying methods, influence enzymatic activity and antioxidant potential. Freeze-drying and hot-air drying better preserve phenolic compounds. Additionally, *chicory* extracts may show both antioxidant and pro-oxidant effects depending on conditions, though heat treatment can reduce pro-oxidant components. Overall, *C. intybus* demonstrates strong antioxidant potential, with applications enhanced through modern formulations such as nanocomposites and nanoparticles (Birsa & Sarbu, 2023).

3.5.3 Anti-Inflammatory Activity

Cichorium intybus is traditionally used externally in the form of compresses to treat various skin conditions, including dermatitis, mucosal inflammation, ulcers, wounds, and trauma. Infusions of its flowers are particularly beneficial for soothing irritated skin and eyes due to their antiseptic, anti-inflammatory, moisturizing, and nourishing properties. They are also used to manage eczema, promote wound healing, improve circulation around the eyes, reduce puffiness, and relieve eye fatigue, while supporting care for dry skin. The plant is often used in combination with other medicinal herbs. Experimental studies have shown that *chicory* root extracts possess significant anti-inflammatory activity, reducing edema and lowering inflammatory markers while enhancing antioxidant defenses. Clinical investigations further suggest that chicory root extracts may be beneficial in managing osteoarthritis, with good tolerability observed in most cases (**Rizvi et al., 2014**).

3.5.4 Antihepatotoxic

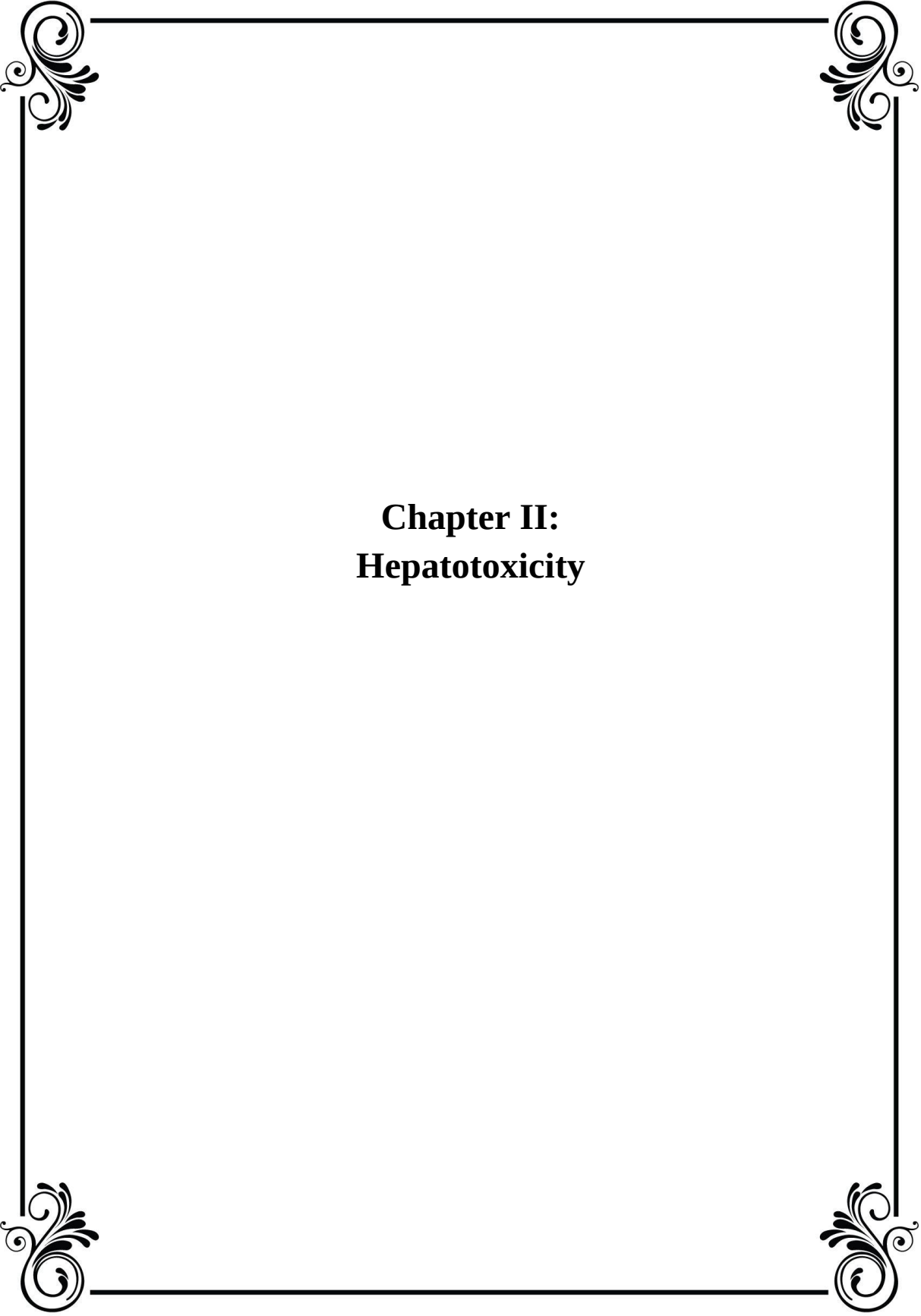
Cichorium intybus roots, seeds, and leaves exhibit strong hepatoprotective and antihepatotoxic properties in animal studies. Both aqueous and alcoholic seed extracts reduce liver damage induced by toxic agents such as carbon tetrachloride, with alcoholic extracts showing greater efficacy. Extracts from roots and callus tissues also demonstrate significant protective effects against hepatic injury. A bioactive compound, cichotyboside, isolated from the seeds, has shown hepatoprotective activity comparable to Silymarin. Additionally, dietary supplementation with *chicory* has been reported to reduce oxidative stress and liver damage caused by nitrosamine compounds. Toxicological studies indicate that *C. intybus* is relatively safe, with no observed toxicity at high doses, suggesting a high margin of safety (**Das et al., 2016**).

3.5.5 Antidiabetic

Cichorium intybus has demonstrated significant antidiabetic potential in experimental studies. Extracts of the plant, particularly ethanolic and aqueous seed extracts, have been shown to reduce blood glucose levels, improve glucose tolerance, and prevent weight loss in diabetic animal models. They also exhibit hypolipidemic effects by lowering triglycerides and cholesterol levels. *Chicory* treatment helps normalize key biochemical parameters, enhances insulin secretion, and improves insulin sensitivity, especially in early-stage diabetes. Additionally, it reduces oxidative stress by decreasing lipid peroxidation and increasing antioxidant defenses such as glutathione and catalase. Bioactive compounds such as caffeic acid, chlorogenic acid, and chicoric acid contribute to these effects by promoting glucose uptake and stimulating insulin release (**Street et al., 2013**).

3.5.6 Other activities

Cichorium intybus exhibits a wide range of biological activities. It shows strong anthelmintic effects, as animals grazing on *chicory* have reduced gastrointestinal parasite burdens, mainly due to compounds such as condensed tannins and sesquiterpene lactones. These compounds also inhibit larval development and egg hatching of parasitic nematodes. *Chicory* additionally demonstrates antimalarial activity, with bitter sesquiterpene compounds effectively inhibiting *Plasmodium falciparum*. It has antiulcerogenic properties, significantly protecting against experimentally induced gastric ulcers. The plant also shows analgesic and mild sedative effects, with certain compounds producing pain relief comparable to standard drugs. Furthermore, *C. intybus* exhibits anticancer potential by inhibiting tumor growth and reducing cancer cell proliferation. It also possesses anti-allergic activity, reducing histamine release and suppressing allergic reactions in experimental models (Street et al., 2013).



Chapter II: Hepatotoxicity

1. Liver

The liver is a critical organ in the human body responsible for an array of functions that help support metabolism, immunity, digestion, detoxification, and vitamin storage, among other functions. It comprises around 2% of an adult's body weight. The liver is unique due to its dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%) **(Kalra et al., 2023)**.

1.1 Anatomy

The liver is located inferior to the diaphragm and occupies most of the right upper quadrant (RUQ) of the abdomen. It is predominantly intraperitoneal and extends from the fifth intercostal space along the midclavicular line to the right costal margin. The superior posterior surface of the liver contains a bare area that is in direct contact with the diaphragm and the inferior vena cava. The remaining surface is covered by visceral peritoneum, which reflects onto the diaphragm at the margins of the bare area to form the coronary ligament. Inferiorly, the liver is closely related to the gallbladder and the right kidney. The falciform ligament divides the liver anatomically into right and left lobes along its anterior surface. Its free edge contains the round ligament (ligamentum teres hepatis), a remnant of the embryonic umbilical vein **(Vernon et al., 2022)**.

Anatomically, the liver consists of four lobes: right, left, caudate, and quadrate. The caudate lobe is bounded medially by the ligamentum venosum, posteriorly by the inferior vena cava, and anteriorly by the porta hepatis **(figure 3)**. The ligamentum venosum itself represents the remnant of the embryonic ductus venosus. The quadrate lobe is situated anterior to the porta hepatis and lateral to the round ligament, maintaining a close anatomical relationship with the gallbladder. Despite these anatomical divisions, the four lobes do not correspond to the liver's eight functional segments, which are classified according to vascular supply and biliary drainage **(Vernon et al., 2022)**.

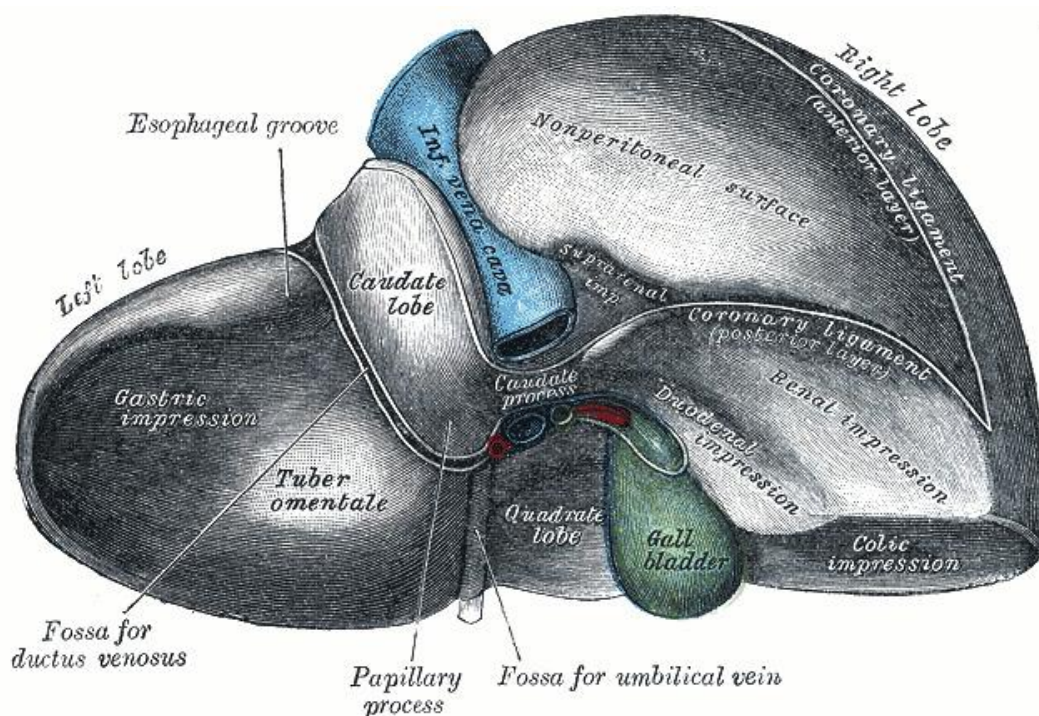


Figure 3: Posterior and Inferior Surfaces of the Liver (Vernon et al., 2022).

The liver is composed of several specialized cell types, including hepatocytes, cholangiocytes, stellate cells, Kupffer cells, and liver sinusoidal endothelial cells, each contributing to hepatic homeostasis. Hepatocytes constitute the major cell population and perform most metabolic, synthetic, and detoxification functions. Cholangiocytes line the bile ducts, while stellate cells store vitamin A in their quiescent state and become activated during liver injury, contributing to collagen deposition and fibrosis. Kupffer cells act as resident macrophages involved in immune responses and tissue repair, whereas sinusoidal endothelial cells facilitate selective exchange between blood and liver cells through their fenestrated structure (Trefts et al., 2017).

Structurally, liver cells are organized into lobules, the functional units of the liver, consisting of hepatocyte cords arranged around a central vein and bordered by portal triads containing branches of the hepatic artery, portal vein, and bile ducts. Blood from the hepatic artery and portal vein flows through sinusoids, creating gradients of oxygen, nutrients, and metabolites across the lobule. These gradients establish metabolic zonation, where hepatocytes in different zones exhibit distinct metabolic functions and adaptive capacities. This complex cellular organization enables the liver to maintain its diverse physiological functions and regenerative capacity (Trefts et al., 2017).

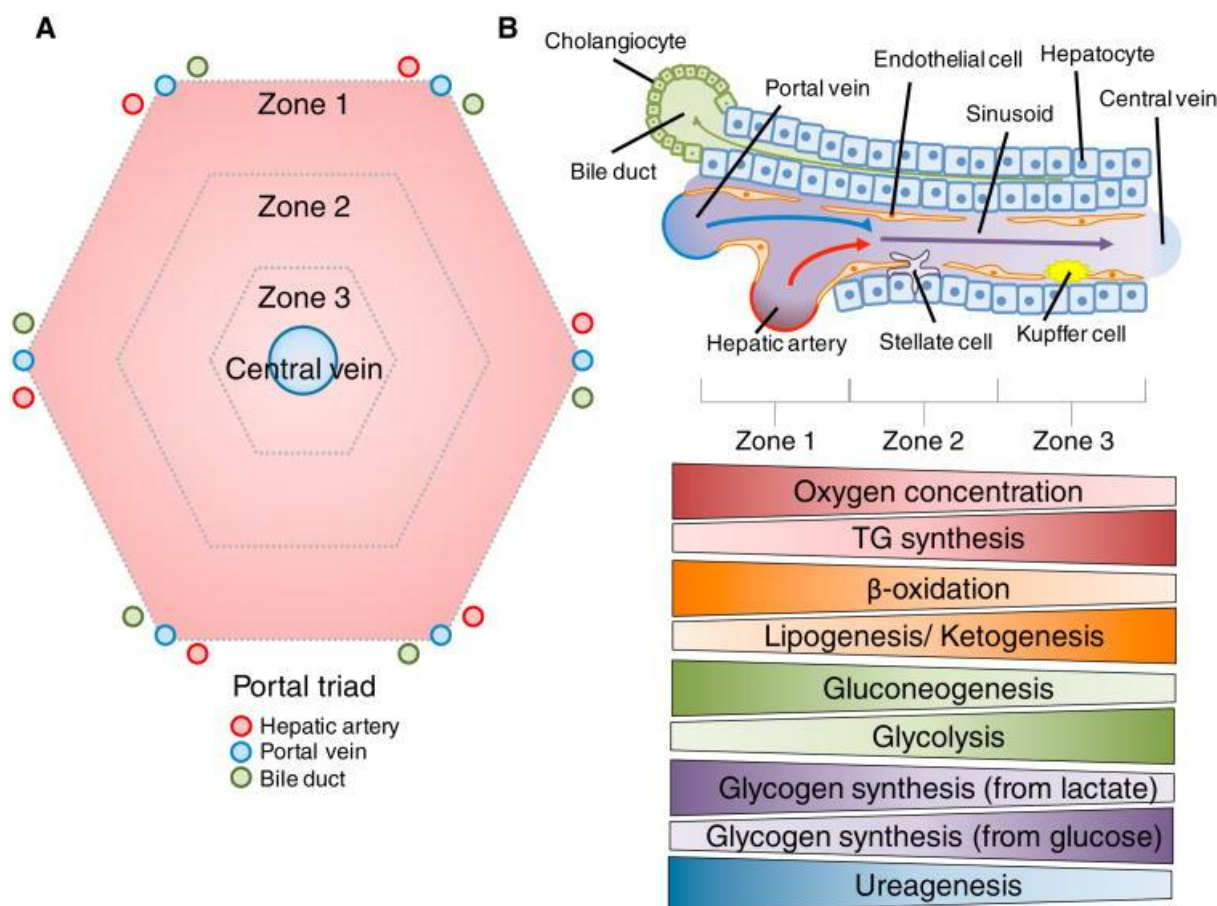


Figure 4: Organization of the liver: (A) Geometric representation of a hepatic lobule, (B) Schematic representation of hepatic sinusoids and metabolic zonation across the liver lobule (Trefts et al., 2017).

1.2 Function

The liver performs multiple essential physiological functions, including bile production, vitamin metabolism, detoxification, bilirubin processing, and protein synthesis. Bile, produced by hepatocytes, facilitates lipid digestion and absorption while serving as a route for excreting waste products. It circulates through the biliary system into the intestine, where many of its components undergo enterohepatic recycling. The liver also regulates the storage and metabolism of fat-soluble vitamins (A, D, E, and K). Vitamin A is stored mainly in stellate cells, vitamin D undergoes hepatic hydroxylation for activation, vitamin E is selectively metabolized and redistributed, and vitamin K is required for the synthesis of coagulation factors (Kalra et al., 2023).

Drug metabolism and detoxification occur primarily through biotransformation reactions in hepatocytes. Phase I reactions, mainly mediated by cytochrome P450 enzymes, convert lipophilic compounds into more reactive intermediates, while Phase II reactions conjugate these metabolites to increase water solubility and facilitate excretion through bile or urine. The liver is also central to bilirubin metabolism, converting unconjugated bilirubin derived from heme degradation into

conjugated bilirubin for biliary excretion. Additionally, it contributes to endocrine regulation by converting thyroid hormone T4 into active T3 and synthesizes most plasma proteins, including albumin, transport proteins, coagulation factors, and anticoagulant proteins (Kalra et al., 2023).

2. Oxidative stress

Oxidative stress is defined as an imbalance between oxidants and antioxidants that favors oxidant accumulation, leading to disturbances in redox signaling, cellular regulation, and potential molecular damage. The concept has evolved beyond its original definition to incorporate the important role of redox signaling in maintaining cellular homeostasis. Under normal physiological conditions, cells maintain a stable redox balance that provides a basal redox state; deviations from this equilibrium trigger adaptive stress responses. This concept also distinguishes between opposite and varying levels of redox imbalance. A shift toward excessive reducing conditions is referred to as reductive stress, whereas oxidative changes can be classified into oxidative eustress and oxidative distress. Oxidative eustress represents physiological and beneficial redox signaling processes essential for normal cellular functions, while oxidative distress refers to excessive oxidative conditions associated with pathological damage. Together, these concepts highlight the importance of redox homeostasis in maintaining cellular function and physiological balance (Sies, 2020).

2.1 Oxidants and Free Radical Production

Reactive oxygen species (ROS) are produced through both enzymatic and nonenzymatic mechanisms. Enzymatic ROS generation mainly occurs during processes such as mitochondrial respiration, prostaglandin synthesis, phagocytosis, and the cytochrome P450 system. Key enzymes including NADPH oxidase, xanthine oxidase, and peroxidases produce superoxide radicals ($O_2^{\bullet-}$), which serve as precursors for other reactive species such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), peroxynitrite ($ONOO^-$), and hypochlorous acid ($HOCl$). Hydrogen peroxide is generated by several oxidases, whereas hydroxyl radicals, among the most reactive ROS, are mainly formed through the Fenton reaction involving (H_2O_2) and transition metals such as iron or copper. Nitric oxide ($NO^{\bullet}$), another reactive molecule with important physiological functions, is synthesized by nitric oxide synthase during arginine oxidation. ROS can also arise through

nonenzymatic processes, including reactions between oxygen and organic molecules, exposure to ionizing radiation, and mitochondrial respiration (**Pizzino et al., 2017**).

Free radicals originate from endogenous and exogenous sources. Endogenous production is associated with physiological and pathological conditions such as inflammation, immune activation, ischemia, infections, cancer, intense physical activity, psychological stress, and aging. Exogenous sources include environmental pollutants, heavy metals, medications, cigarette smoke, alcohol, chemical solvents, dietary factors, and radiation exposure. These external agents often generate free radicals as metabolic by-products after entering the body (**Pizzino et al., 2017**).

2.2 Oxidative Stress in the Pathogenesis of Disease

Oxidative stress is a major contributor to the development of metabolic syndrome, cellular senescence, and cancer through excessive production of reactive oxygen species (ROS), which disrupt cellular homeostasis and promote chronic inflammation. In metabolic syndrome, oxidative stress drives obesity-related inflammation, insulin resistance, β -cell dysfunction, endothelial impairment, and cardiovascular complications by altering mitochondrial function and metabolic signaling pathways. Persistent ROS accumulation also induces cellular senescence by causing DNA damage, activating cell-cycle arrest mechanisms, and promoting the secretion of pro-inflammatory mediators associated with the senescence-associated secretory phenotype (SASP), which contributes to aging, neurodegenerative disorders, osteoarthritis, and osteoporosis. In cancer, oxidative stress promotes genomic instability, tumor initiation, proliferation, angiogenesis, and metastasis through ROS-mediated signaling pathways. However, ROS exert a dual effect, as excessive accumulation may also trigger different forms of programmed cancer cell death, including apoptosis, ferroptosis, necroptosis, and pyroptosis (**Qin et al., 2026**).

2.3 Antioxidant Defense Mechanisms in the Liver

Liver cells possess efficient antioxidant defense systems that maintain reactive oxygen species (ROS) at physiological levels through both enzymatic and non-enzymatic mechanisms. Enzymatic antioxidants, including SOD, GPX, GRX, PRX, TRX, and CAT, neutralize ROS directly, whereas non-enzymatic antioxidants such as glutathione, bilirubin, coenzyme Q10, uric acid, vitamins, and dietary compounds like flavonoids help

limit oxidative damage by scavenging free radicals. Antioxidant defense also involves reducing ROS production through inhibition of pro-oxidant enzymes and enhancing endogenous antioxidant activity. A central regulator of this protective network is the NRF2/ARE signaling pathway, which controls cellular antioxidant responses. Under normal conditions, NRF2 remains bound to Keap1 in the cytoplasm and undergoes degradation; however, oxidative stress triggers NRF2 release and translocation into the nucleus, where it activates antioxidant response elements (AREs). This process induces the expression of antioxidant enzymes, detoxifying proteins, and stress-response genes, thereby strengthening cellular defenses and maintaining liver homeostasis (Allameh et al., 2023).

2.4 The Impact of Oxidative Stress on Liver Cells

Oxidative stress plays a central role in the initiation and progression of liver diseases by disrupting redox homeostasis through excessive production of reactive oxygen and nitrogen species (ROS/RNS). Various factors, including metabolic disorders, alcohol consumption, viral infections, toxins, drugs, and environmental exposures, enhance oxidative damage, leading to inflammation, fibrosis, apoptosis, necrosis, and carcinogenesis. Hepatocytes, particularly their mitochondria, are major sources and targets of ROS, where mitochondrial dysfunction, lipid peroxidation, calcium imbalance, and impaired energy metabolism amplify oxidative injury and promote cell death. The release of damage-associated molecular patterns (DAMPs) activates non-parenchymal liver cells, including Kupffer cells (KCs), hepatic stellate cells (HSCs), and immune cells, further enhancing inflammatory and fibrotic responses. Oxidative stress also stimulates HSC activation and extracellular matrix deposition, contributing to fibrosis, cirrhosis, and hepatocellular carcinoma development. In addition, oxidative damage affects liver sinusoidal endothelial cells (LSECs), cholangiocytes, and immune cells by impairing vascular function, bile secretion, autophagy, and immune responses, ultimately promoting liver dysfunction and disease progression (Allameh et al., 2023).

3. Liver Toxicity

Liver diseases encompass a broad spectrum of disorders characterized by hepatocellular damage, inflammatory cell infiltration, and activation of hepatic stellate cells (HSCs), leading to progressive impairment of liver function and structural alterations.

Globally, liver diseases are responsible for nearly 2 million deaths annually, accounting for approximately 4% of total mortality. Acute liver diseases are primarily associated with infections caused by hepatotropic viruses, although drug-induced liver injury (DILI) has emerged as an increasingly important cause worldwide. In contrast, chronic liver diseases commonly result from excessive alcohol intake, chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), as well as the growing prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD). The progression of chronic liver disorders to advanced stages, such as cirrhosis and liver cancer, represents a major contributor to global morbidity and mortality (**Gan et al., 2025**).

3.1 Epidemiology

Liver diseases remain a major global health burden and a leading cause of mortality, with increasing incidence and deaths worldwide driven by chronic liver conditions, cirrhosis, and liver cancer. The epidemiology of liver disease has shifted from predominantly viral causes toward lifestyle-associated disorders, particularly MASLD and alcohol-associated liver disease (ALD), fueled by obesity, diabetes, sedentary lifestyles, and increasing alcohol consumption. Although preventive strategies, vaccination programs, screening, and antiviral therapies have reduced new infections of viral hepatitis, chronic infections with HBV and HCV continue to contribute substantially to liver-related morbidity and mortality, especially in low- and middle-income regions. Acute liver diseases remain associated with viral hepatitis, particularly HAV and HEV, while DILI is becoming increasingly prevalent, partly due to growing use of medications and herbal products. Chronic liver diseases—including viral hepatitis, MASLD, ALD, autoimmune disorders, and genetic conditions—often progress silently toward fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Significant geographic disparities exist in prevalence and mortality due to differences in healthcare access, socioeconomic conditions, environmental exposures, lifestyle factors, and public health policies. The increasing prevalence of end-stage liver diseases and hepatobiliary cancers highlights the urgent need for region-specific prevention strategies, early detection programs, and improved therapeutic interventions (**Gan et al., 2025**).

3.2 Etiology

Liver diseases arise from multiple etiological factors, including infections, metabolic disturbances, toxic exposures, and genetic predisposition. Viral infections remain major contributors, with HBV, HCV, HDV, HAV, and HEV causing acute and chronic liver diseases that may progress to cirrhosis and hepatocellular carcinoma through persistent infection, immune dysregulation, and hepatocyte injury. Parasitic infections also contribute to liver pathology by inducing fibrosis, portal hypertension, and tissue destruction. Metabolic stress, particularly associated with obesity, insulin resistance, type 2 diabetes, and metabolic dysfunction, has driven the rapid emergence of MASLD, which can progress from steatosis to cirrhosis and cancer. Toxic exposures, including medications, alcohol, industrial chemicals, environmental toxins, and mycotoxins, represent another major cause of liver injury, with DILI and ALD increasingly contributing to acute and chronic liver damage. In addition, inherited disorders such as hereditary hemochromatosis, Wilson disease, and alpha-1 antitrypsin deficiency promote liver injury through abnormal metal metabolism, oxidative stress, protein accumulation, and chronic cellular damage, highlighting the complex interplay between genetic and environmental factors in liver disease pathogenesis (**Gan et al., 2025**).

3.3 Hepatotoxicity by Drugs

DILI is a major cause of liver dysfunction and can manifest with a broad spectrum of clinical presentations, ranging from asymptomatic elevation of liver enzymes and mild hepatitis to cholestasis, chronic liver disease, acute liver failure, and severe hepatic damage. Numerous drug classes are implicated in DILI, including nonsteroidal anti-inflammatory drugs (NSAIDs), anti-infective agents, anticancer drugs, hormonal therapies, immunosuppressants, sedatives, and neuropsychiatric medications. Acetaminophen is the most frequently associated drug overall, whereas antibiotics are the leading class responsible for hepatotoxicity, with amoxicillin-clavulanate being the most commonly implicated agent. Idiosyncratic DILI is predominantly linked to antibiotics, followed by herbal and dietary supplements, cardiovascular medications, central nervous system drugs, and antineoplastic agents. The increasing use of herbal products and multi-drug therapies further contributes to the growing burden and complexity of DILI diagnosis and management (**Bashir et al., 2023**).

3.3.1 Pathophysiology et Histopathology

The pathogenesis of DILI involves two major mechanisms: intrinsic and idiosyncratic toxicity. Intrinsic DILI is predictable, dose-dependent, and reproducible, generally occurring after a short latency period following exposure to hepatotoxic drugs. A classic example is acetaminophen toxicity, where excessive accumulation of reactive metabolites induces oxidative stress, hepatocyte apoptosis, and necrosis. In contrast, idiosyncratic DILI is less predictable, usually dose-independent, and occurs only in susceptible individuals after variable exposure periods, often within days to weeks after treatment initiation. Its development is influenced by complex interactions between host-related factors (age, sex, genetics, immune status, and metabolism), drug characteristics (dose, duration, molecular weight, and lipophilicity), and environmental factors such as alcohol consumption, diet, smoking, and toxin exposure. Mechanistically, idiosyncratic DILI may occur through immune-mediated hypersensitivity reactions or through non-immune pathways involving metabolic disturbances and mitochondrial dysfunction **(Francis & Navarro, 2024)**.

Although liver biopsy is not routinely required for the diagnosis of DILI, histopathological evaluation can provide valuable information for identifying injury patterns, determining potential etiologies, excluding alternative diagnoses, guiding management, and assessing prognosis. Histological manifestations of DILI are heterogeneous and include acute hepatocellular injury characterized by inflammation, necrosis, and apoptosis; chronic hepatocellular injury associated with fibrosis and features resembling chronic liver disease; acute and chronic cholestatic patterns marked by bile accumulation and duct injury; steatosis, often linked to mitochondrial dysfunction; and zonal necrosis, which is typically associated with intrinsic toxicity and poor prognosis, particularly in acetaminophen overdose. Additionally, granulomatous lesions may occur in milder forms of injury and can result from drug exposure or other exogenous substances. These diverse histological patterns reflect the complexity of DILI and its variable clinical presentation **(Francis & Navarro, 2024)**.

3.3.2 Evaluation

The diagnosis of DILI primarily relies on obtaining a compatible clinical history, identifying exposure to potentially hepatotoxic drugs, and excluding preexisting liver

diseases or alternative causes of hepatic injury, as no specific diagnostic test currently exists. Laboratory evaluation typically includes liver function tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin, albumin, prothrombin time (PT), and international normalized ratio (INR), along with additional investigations including complete blood count, electrolyte analysis, viral serologies, and autoimmune markers. The pattern of biochemical abnormalities helps classify liver injury into hepatocellular, cholestatic, or mixed forms based on the relative elevation of transaminases and ALP levels. In severe cases, particularly acute liver failure, coagulation abnormalities may indicate impaired hepatic function. Imaging techniques such as ultrasound or magnetic resonance imaging are useful, especially in cholestatic presentations, to exclude biliary obstruction or structural abnormalities. Although liver biopsy is not routinely required, it may aid in differential diagnosis when other liver diseases are suspected. Furthermore, emerging biomarkers, including circulating microRNAs released from damaged hepatocytes, show potential for improving DILI diagnosis and monitoring disease progression, although their specificity remains limited **(Francis & Navarro, 2024)**.

3.3.3 Categories of Hepatotoxicity

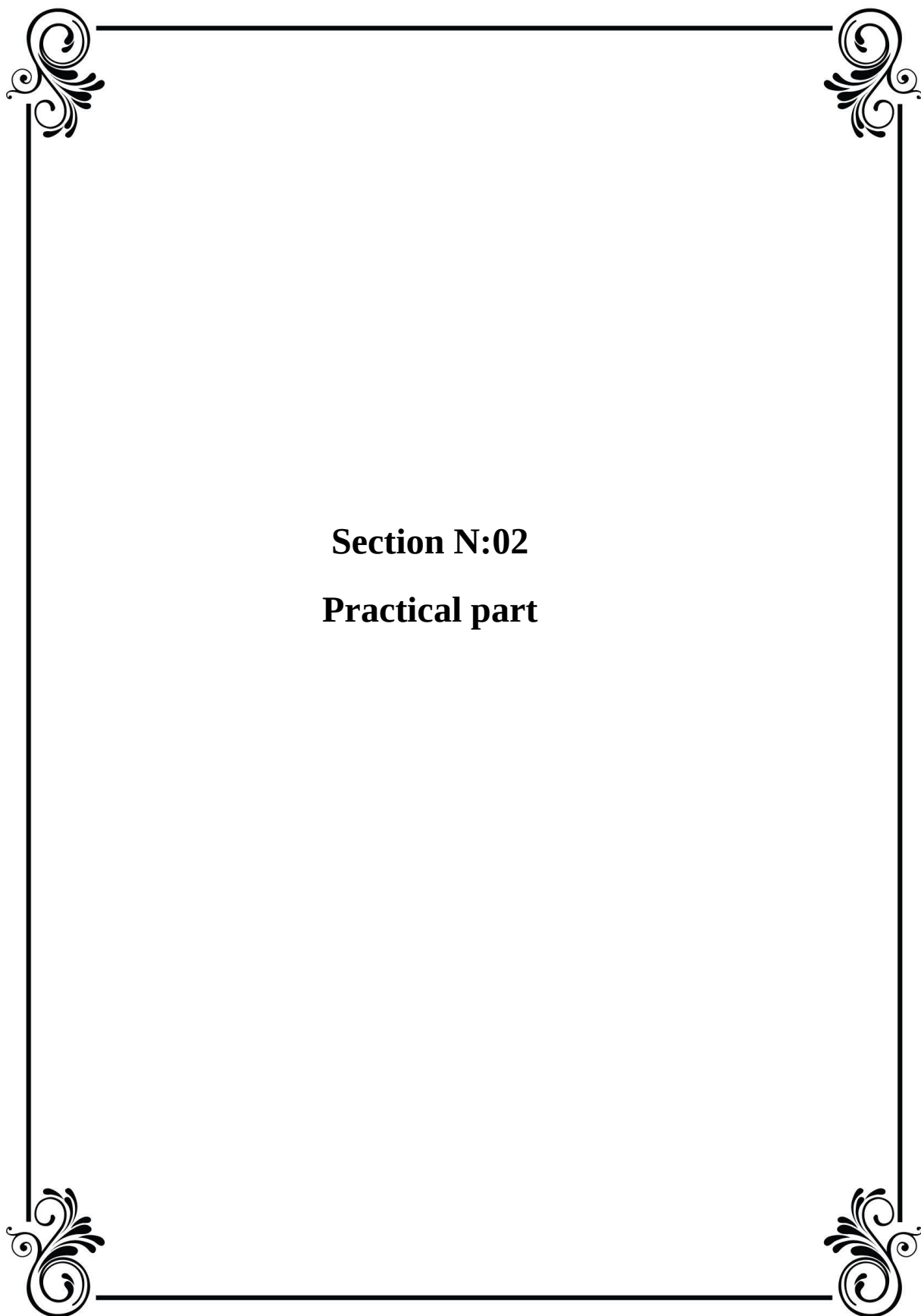
LiverTox classifies drugs according to their likelihood of causing DILI based on published evidence. Drugs were categorized into groups A to D according to the number of reported hepatotoxicity cases, with category A including drugs with the strongest evidence (>50 reports) and category D containing drugs with only a few reported cases. An additional category (T) was introduced for drugs causing hepatotoxicity mainly at supratherapeutic doses, while category E included drugs without convincing evidence of liver toxicity. Analysis of 671 drugs showed that only 53% had documented evidence of hepatotoxicity, whereas nearly half lacked sufficient evidence despite frequent mention of liver injury in product labeling. Drugs with stronger evidence of hepatotoxicity (categories A and B) were generally older medications with more reported fatal cases and positive rechallenge events, supporting their established hepatotoxic potential. In contrast, the causal relationship remains less certain for drugs in categories C and D because of the limited number of reported cases. These findings highlight the discrepancy between reported adverse effects and documented evidence, emphasizing the need for careful evaluation of hepatotoxic risk **(Björnsson, 2016)**.

Table 6: Examples of drugs classified in categories A and B (Björnsson, 2016).

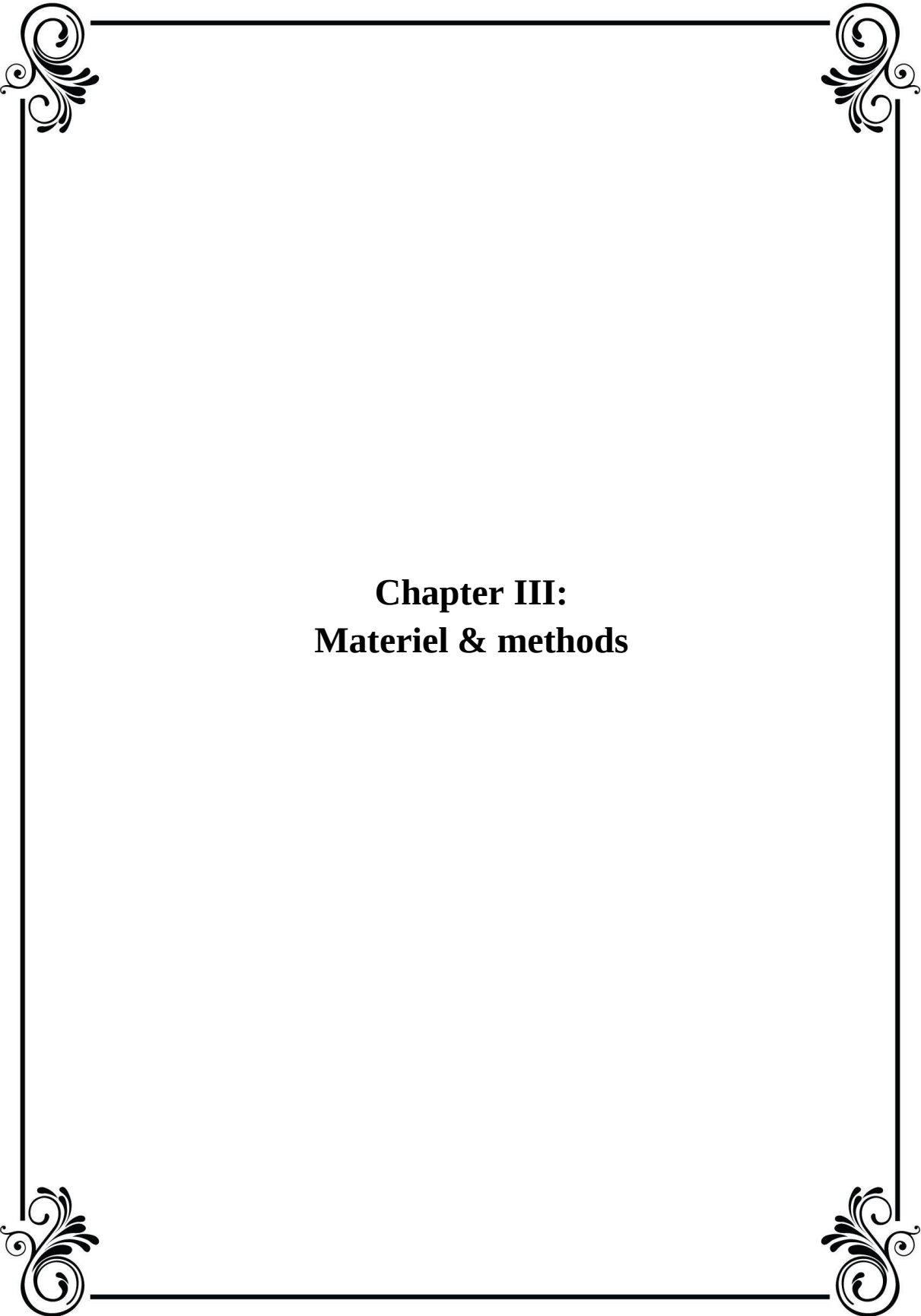
Categories	Drug	Drug Class/Indication
Category A	Allopurinol Amoxicillin-clavulanate Carbamazepine Diclofenac Erythromycin Ibuprofen Sulfonamides	Gout prophylaxis Antibiotic Antiepileptic NSAID Antimicrobial NSAID Antibiotic
Category B	Chlorzoxazone Cyproterone Heparin Oxacillin Terbinafine	Muscle relaxant Antineoplastic Anticoagulant Antimicrobial HIV

3.3.4 Hepatoprotective herbal medicines

Medicinal herbs have long been used in the management of liver diseases due to their therapeutic potential and diverse biological activities. Numerous plants contain bioactive compounds with hepatoprotective properties, although only a limited number have been extensively investigated regarding their active constituents and mechanisms of action. Understanding the hepatoprotective effects of medicinal plants is important for both clinical practice and research, particularly because herbal medicines are often considered more affordable, associated with fewer adverse effects, and generally better tolerated than conventional therapies. Several medicinal plants, including *Silybum marianum*, *Glycyrrhiza glabra*, *Phyllanthus amarus*, *Salvia miltiorrhiza*, *Astragalus membranaceus*, *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Ginkgo biloba*, *Schisandra chinensis*, *Lycium barbarum*, and others, have demonstrated promising hepatoprotective effects in experimental studies. Their mechanisms of action are diverse and include antioxidant activity through enhancement of endogenous antioxidant enzymes, reduction of reactive oxygen species, immunomodulatory effects, stimulation of hepatic regeneration, inhibition of viral replication, suppression of lipid accumulation, free radical scavenging, and attenuation of hepatic fibrosis through modulation of profibrotic pathways (Ali et al.,2018).



Section N:02
Practical part



Chapter III: Materiel & methods

1. The objective

This study was conducted to evaluate the protective effects of *Anacyclus pyrethrum*, *Cichorium intybus*, and their combination against experimentally induced hepatotoxicity in rats. The assessment was performed through biochemical analyses, evaluation of oxidative stress markers, and histopathological examination of liver tissues in order to investigate the potential hepatoprotective and antioxidant properties of these medicinal plants.

2. Preparation of Plant Extracts

The collected samples ground to a fine powder using an electric blender, then 10 g of the dried powder was extracted by maceration with 1000 mL of 70% ethanol at room temperature for 48 h. Extracted contents were filtered and concentrated on a rotary evaporator to reduce the volume. On complete drying of the solvent using a fume cupboard for 24 h; the obtained yield was measured by applying Equation below. The obtained extracts were stored at 4°C in sealed glass vials until their administration to the experimental rats (Jawhari et al.,2020; Javeed et al 2022).

3. Experimental Animals

The experiments were carried out on Wistar rats weighing between 210 and 350 g, obtained from the Department of Animal Facilities at the Pasteur Institute of Algiers. The animals were then housed in the animal facility of the Biology Department at the University of Saïda and allowed to acclimatize for more than one week before the beginning of the experimental study. The rats were maintained in a well-ventilated room under controlled conditions at a temperature of $21 \pm 1^{\circ}\text{C}$. They were kept in Macrolon cages ($40 \times 25 \times 18$ cm) with free access to standard rodent pellets and tap water throughout the experiment.

4. Distribution of Experimental Groups

A total of 40 rats were divided into eight groups, with each group containing five rats. Preventive treatments were administered for seven consecutive days, and acute hepatotoxicity was induced on the eighth day by paracetamol administration. Distilled

water, paracetamol, and plant extracts were administered orally by gavage.

- **Group 1 (Control group):** received distilled water for 8 days.
- **Group 2 (Intoxicated group):** received distilled water for 7 days, followed on the 8th day by a single dose of paracetamol (3 g/kg BW) (Henneh et al., 2022; Teixeira et al., 2025).
- **Groups 3, 4, and 5 (Treated control groups):** received *Anacyclus pyrethrum* extract (200 mg/kg BW, p.o.) (Deshpande et al., 2010), *Cichorium intybus* extract (200 mg/kg BW, p.o.) (Khalid et al., 2018; Moloudi et al., 2021), and a mixture of both plants (100 mg/kg BW, p.o. each), respectively, for 8 days.
- **Groups 6, 7, and 8 (Pretreated intoxicated groups):** received preventive treatment with *Anacyclus pyrethrum* extract (200 mg/kg BW, p.o.), *Cichorium intybus* extract (200 mg/kg BW, p.o.), or their mixture (100 mg/kg BW, p.o. each) for 7 days, followed on the 8th day by acute intoxication with paracetamol (3 g/kg BW).

5. Sample collection

At the end of the experimental period, the rats were fasted for 12 hours and then sacrificed under anesthesia followed by decapitation. Blood samples were collected in heparinized tubes for biochemical analyses. The liver tissues were carefully excised, washed with physiological saline solution (0.9% NaCl), dried, and weighed. Subsequently, some liver tissues were fixed in 10% diluted formaldehyde for histological analysis, while the remaining tissues were used for the evaluation of oxidative stress parameters.

6. Biochemical analysis

6.1 Transaminase Assays

- **Principle**

The determination of transaminase activity, namely aspartate aminotransferase (AST/TGO) and alanine aminotransferase (ALT/TGP), is based on a colorimetric method. AST catalyzes the reaction between aspartate and α -ketoglutarate to produce oxaloacetate and glutamate, whereas ALT catalyzes the reaction between alanine and α -ketoglutarate to form pyruvate and glutamate. The pyruvate or oxaloacetate formed reacts with 2,4-

dinitrophenylhydrazine (DNPH) to produce colored hydrazone derivatives. The intensity of the developed color is measured spectrophotometrically at 505 nm and is proportional to the enzyme activity present in the serum sample (**Reitman & Frankel, 1957; Vroon & Israili, 1990**). **Colorimetric determination of AST (TGO) and ALT (TGP) activities according to the following reactions:**

AST (TGO):

Aspartate + α -ketoglutarate $\rightarrow$ Oxaloacetate + Glutamate

ALT (TGP):

Alanine + α -ketoglutarate $\rightarrow$ Pyruvate + Glutamate

- **Operating Procedure**

For the assay, 1 mL of AST (Reagent 1) or ALT substrate (Reagent 2) was introduced into test tubes and pre-incubated at 37°C for 5 minutes. Then, 0.2 mL of serum sample was added, mixed thoroughly, and incubated at 37°C for 1 hour for AST determination and 30 minutes for ALT determination. After incubation, 1 mL of DNPH reagent (Reagent 3) was added and the mixture was allowed to stand for 20 minutes at room temperature. Subsequently, 10 mL of 0.4 N sodium hydroxide solution was added to each tube. After 5 minutes, the absorbance was measured at 505 nm against distilled water as blank. The enzyme activities were determined using a standard calibration curve and expressed as units per milliliter of serum (**Reitman & Frankel, 1957**).

6.2 Bilirubin Assay

- **Principle**

Bilirubin is converted into azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions present in serum, bilirubin glucuronide and free bilirubin bound to albumin, only the first reacts in an aqueous medium (direct bilirubin). The second reacts only after solubilization with dimethyl sulfoxide (DMSO) (indirect bilirubin). In the determination of indirect bilirubin, direct bilirubin is also measured; therefore, the obtained result corresponds to total bilirubin. The intensity of the color formed is proportional to the bilirubin concentration present in the tested sample (**Kalakonda et al., 2017**).

- **Operating Procedure**

Total and direct bilirubin levels were determined by a colorimetric method according to the **Jendrassik-Grof** method (1938). Before analysis, all reagents were brought to room temperature. Four test tubes were prepared: two for total bilirubin determination and two for direct bilirubin determination, including blank and test samples. For total bilirubin assay, 1.5 mL of Total Bilirubin Reagent (R1) was mixed with serum sample and sodium nitrite reagent (R3). For direct bilirubin assay, 1.5 mL of Direct Bilirubin Reagent (R2) was used instead of R1. The reaction mixtures were thoroughly mixed and incubated for exactly 5 minutes at 15–20°C. Absorbance was then measured at 555 nm against distilled water as blank using a spectrophotometer. Bilirubin concentrations were calculated using a calibration factor and expressed in mg/dL.

6.3 Alkaline Phosphatase Assay

- **Principle**

Alkaline phosphatase (ALP) activity was determined using a colorimetric method based on the hydrolysis of p-nitrophenylphosphate (pNPP). In the presence of magnesium ions, ALP catalyzes the conversion of pNPP into phosphate and p-nitrophenol. The released p-nitrophenol produces a yellow color in alkaline medium, and its intensity is directly proportional to the ALP activity in the sample. The absorbance was measured spectrophotometrically at 450 nm (**Bablok et al., 1988**).



- **Operating Procedure**

ALP activity was measured according to the manufacturer's instructions using a commercial diagnostic kit. Briefly, the reagent was brought to room temperature before use. An appropriate volume of reagent was mixed with the serum sample in test tubes and incubated under controlled conditions. After incubation, the enzymatic reaction led to the formation of p-nitrophenol, whose absorbance was measured at 450 nm using a spectrophotometer against a reagent blank. The ALP activity was then calculated and expressed in U/L (**Bablok et al., 1988**).

6.4 Gamma glutamyl transferase (GGT) assay

- **Principle**

The Gamma-Glutamyl Transferase (GGT) assay is based on a **kinetic colorimetric method**. GGT catalyzes the transfer of the γ -glutamyl group from a synthetic substrate, L- γ -glutamyl-3-carboxy-4-nitroanilide, to an acceptor molecule, glycylglycine. This enzymatic reaction leads to the formation of **p-nitroaniline**, a colored compound. The rate of formation of p-nitroaniline is directly proportional to the GGT activity present in the sample. The intensity of the color produced is measured spectrophotometrically at **405 nm** (Szasz, 1969).

- **Operating Procedure**

In practice, the assay is performed by first preparing the working reagent by mixing the buffer and substrate provided in the kit. A serum or plasma sample is then added to this reagent mixture. The reaction is carried out at 37°C under standardized conditions. The absorbance is then measured continuously at 405 nm over a defined period using a spectrophotometer. The assay is performed as a kinetic test, where the change in absorbance per minute ($\Delta A/\text{min}$) is recorded. This rate is used to calculate the GGT activity, which is expressed in U/L, using a calibration factor supplied by the manufacturer (Szasz, 1969).

6.5 Total Protein Assay

- **Principle**

Total plasma proteins are measured using the Biuret colorimetric method. In an alkaline medium, copper ions (Cu^{2+}) react with the peptide bonds of proteins to form a violet (purple) copper-protein complex. The intensity of this color is directly proportional to the number of peptide bonds, and therefore proportional to the total protein concentration in the sample. Potassium sodium tartrate is added to stabilize the copper ions in solution and prevent precipitation of copper hydroxide, while potassium iodide prevents the autoreduction of copper. The absorbance of the formed complex is measured at 552 nm, and it is directly proportional to the protein concentration (Weichselbaum, 1946).

- **Operating Procedure**

In practice, the assay is performed by adding a plasma sample to a Biuret reagent containing copper sulfate in alkaline solution, along with stabilizing agents such as potassium sodium tartrate and potassium iodide. The mixture is incubated for a defined period at room temperature or 37°C depending on the protocol, allowing the reaction between copper ions and peptide bonds to occur and form the colored complex. After incubation, the absorbance of the solution is measured using a spectrophotometer at 552 nm against a reagent blank. The intensity of the color produced is then compared to that of a standard solution with a known protein concentration. Using this calibration, the total protein concentration in the sample is calculated and expressed in g/L (**Weichselbaum, 1946**).

7. Determination of oxidative stress markers

7.1 Preparation of liver tissue homogenate

The tissue homogenate was prepared by first weighing 1 g of tissue, which was then homogenized in 5 mL of TBS buffer (Tris 50 mM, NaCl 150 mM, pH 7.4) to obtain a uniform suspension. The homogenization process ensures the release of intracellular components involved in oxidative stress analysis. The resulting suspension was then centrifuged at 9000 rpm for 15 minutes at 4°C (or alternatively at 4500 rpm for 30 minutes) in order to separate cellular debris from the soluble fraction. After centrifugation, the clear supernatant was carefully collected. Finally, the supernatant was aliquoted into Eppendorf tubes and stored at -20°C until further analysis of oxidative stress parameters (**Sundar et al., 2010**).

7.2 Bradford Protein Assay

- **Principle**

The Bradford assay is a colorimetric method used for protein quantification. It is based on the binding of Coomassie Brilliant Blue G-250 dye to proteins in an acidic medium. When the dye binds mainly to basic and aromatic amino acid residues, it undergoes a shift in color from brownish-red to blue. The intensity of the blue color formed is directly

proportional to the protein concentration in the sample. The absorbance is measured at 595 nm (Kielkopf et al., 2020).

- **Operating Procedure**

The Coomassie Brilliant Blue reagent is first prepared by dissolving 100 mg of Coomassie Brilliant Blue in 50 mL of 95% ethanol with continuous stirring for 2 hours. Then, 100 mL of 85% orthophosphoric acid is added and mixed thoroughly. The solution is completed to a final volume of 1 L with distilled water, stirred for a few minutes, filtered if necessary, and stored at 4°C protected from light. A standard stock solution is prepared using bovine serum albumin (BSA), where 1 mg of BSA is dissolved in 1 mL of distilled water. A calibration curve is then established using a series of standard tubes containing increasing volumes of BSA (0–100 µL) completed with distilled water to 100 µL, followed by the addition of 4 mL of Bradford reagent to each tube (Kielkopf et al., 2020).

For sample analysis, 100 µL of supernatant of tissue homogenate is mixed with 4 mL of Bradford reagent. A blank is prepared using 100 µL of distilled water with 4 mL of reagent. All tubes are vortexed and incubated at room temperature for 5–10 minutes to allow color development. Finally, absorbance is measured at 595 nm, and protein concentration is determined by comparison with the standard calibration curve (Kielkopf et al., 2020).

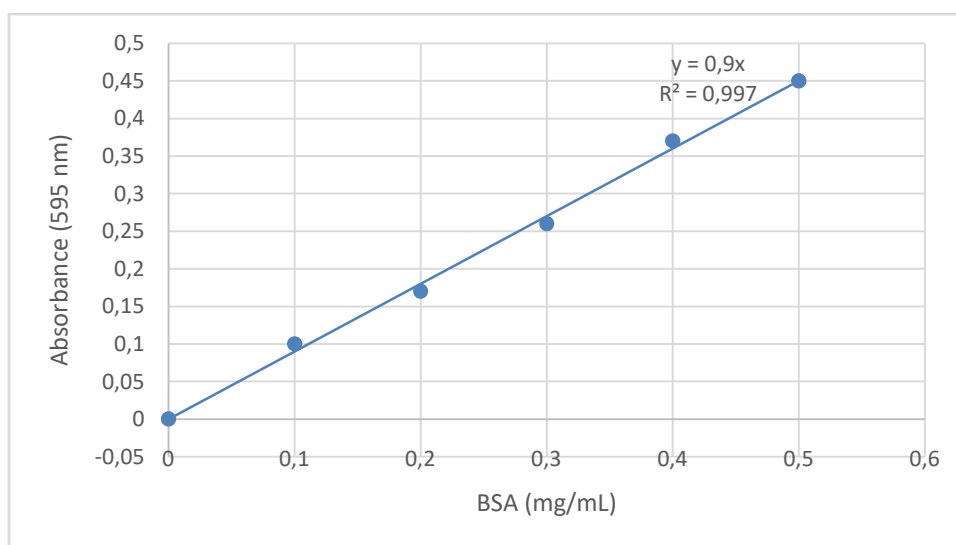


Figure 5: Standard calibration curve

7.3 Catalase activity assay

- **Principle**

The catalase (CAT) activity assay is based on the ability of the enzyme catalase to decompose hydrogen peroxide (H_2O_2) into water and oxygen. In this spectrophotometric method, the decrease in absorbance of H_2O_2 is monitored at 240 nm, since hydrogen peroxide absorbs ultraviolet light at this wavelength. The rate of decrease in absorbance is directly proportional to catalase activity in the sample and is expressed relative to protein content (Montejo et al., 2025).

- **Operating Procedure**

The assay is performed using a reaction mixture containing phosphate buffer (PBS, 0.1 M, pH 7.4), hydrogen peroxide (0.5 M), and tissue homogenate. For the test sample, 780 μ L of PBS is mixed with 200 μ L of H_2O_2 and 20 μ L of homogenate, while the blank contains 800 μ L of PBS and 200 μ L of H_2O_2 without sample. The spectrophotometer is first zeroed using PBS. The reaction is initiated by adding the homogenate, and the decrease in absorbance is measured at 240 nm after 15 seconds and 60 seconds (Montejo et al., 2025).

- **Calculation**

The catalase activity is calculated from the rate of H_2O_2 decomposition using the change in absorbance per minute ($\Delta DO/min = DO_{15s} - DO_{60s}$). The activity is expressed as μ mol of H_2O_2 decomposed per minute per mg of protein (Montejo et al., 2025), using the formula:

$$\text{CAT activity} = \frac{\Delta DO * 10}{\epsilon \cdot L \cdot X}$$

Where:

- ϵ is the molar extinction coefficient of H_2O_2 ($0.0436 \text{ mM}^{-1} \text{ cm}^{-1}$).
- **L:** is the cuvette path length (1 cm).
- **X:** is the protein concentration (mg/mL) determined by Bradford assay.

7.4 Malondialdehyde assay

- **Principle**

The malondialdehyde (MDA) assay is commonly used to evaluate lipid peroxidation, an indicator of oxidative stress. MDA reacts with thiobarbituric acid (TBA) under high temperature and acidic conditions to form a pink MDA–TBA adduct (thiobarbituric acid reactive substances, TBARS complex). The intensity of the colored complex is proportional to the MDA concentration in the sample and is measured spectrophotometrically at 532 nm (**Ohkawa et al., 1979**).

- **Operating Procedure**

For the assay, 375 µL of tissue homogenate was first collected and mixed with 150 µL of TBS buffer (Tris 50 mM, NaCl 150 mM, pH 7.4). Then, 375 µL of precipitation solution containing TCA (20%) and BHT (1%) was added. The mixture is vortexed and centrifuged at 3000 rpm for 10 minutes. After centrifugation, 400 µL of the supernatant is carefully collected. To this supernatant, 80 µL of 0.6 M HCl was added, followed by 320 µL of Tris-TBA reagent (Tris 26 mM, TBA 120 mM). The mixture is homogenized and incubated in a water bath at 80°C for 10 minutes. After incubation, the tubes are cooled to room temperature. The absorbance of the resulting pink chromogen is then measured at 532 nm, using a blank prepared by replacing the sample with distilled water (**Ohkawa et al., 1979**).

- **Calculation**

The MDA concentration is calculated using the following formula:

$$\text{MDA (nmol/mg protein)} = \frac{DO \cdot 10^6}{\epsilon \cdot L \cdot X \cdot Fd}$$

Where:

- **DO** = optical density (absorbance)
- **ε** = molar extinction coefficient of MDA = $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$
- **L** = optical path length 1 cm
- **X** = protein concentration of the extract
- **Fd** = dilution factor (0.2 or 1 if no dilution)

8. Histological Processing of Liver Tissue

- **Principle**

Histology deals with the study of the microscopic structure of cells and tissues of organisms. The knowledge of biological (microscopic) structures and their functions at the sub-cellular, cellular, tissue and organ levels is central to the study of disease proliferation and prognosis of disease. Also to study and analyze histological image under microscope, pathologists identify the morphological characteristics of tissue which indicates the presence of disease like cancer. The biopsy sample is processed and its sections are placed onto glass slides to observe them under microscope for analysis. Pathologist examine the tissue slides under a microscope and observe it at various magnification levels such as 10X, 20X, 40X, 100X etc. to view cells, glands, nucleus, and detects the resemblance of these structures with normal vs. diseased tissue. If the disease detects the grading process is performed which deals with spreading of infected cells all over the tissue. Then for each patient the prognosis and further treatment is planned by considering grade of disease (Belsare & Mushrif, 2012).

- **Operating Procedure**

a. Tissue Collection (Dissection): After sacrifice of the laboratory rats, liver tissues were carefully excised to avoid mechanical damage. Small tissue fragments measuring approximately 0.5–1 cm were collected to ensure proper fixation and adequate penetration of reagents.

b. Fixation: The collected liver samples were immediately immersed in 10% neutral buffered formalin. This step preserves tissue architecture by preventing autolysis and tissue degradation while maintaining the structural integrity of hepatocytes and other cellular components.

c. Tissue Processing and Embedding: Following fixation, the tissues were dehydrated using increasing concentrations of alcohol, then cleared with xylene and infiltrated with molten paraffin wax. The samples were subsequently embedded in paraffin blocks to provide support for thin sectioning.

d. Sectioning: The paraffin-embedded tissues were sectioned into thin slices of approximately 3–5 μm thickness using a microtome. Thin sections are necessary for proper microscopic examination.

e. Water Bath Treatment: The tissue sections were floated on a warm water bath to flatten them and eliminate wrinkles. This step facilitates uniform spreading of the sections before mounting.

f. Mounting and Drying: The flattened sections were carefully mounted onto clean glass slides and left to dry to ensure proper adhesion of the tissue to the slides.

i. Staining: The slides were stained using the standard Hematoxylin and Eosin (H&E) staining method. Hematoxylin stains the nuclei blue-purple, while eosin stains the cytoplasm and extracellular matrix pink, allowing clear visualization of liver tissue morphology.

j. Microscopic Examination: The stained sections were examined under a light microscope to evaluate histopathological alterations such as hepatocellular necrosis, fatty degeneration (steatosis), inflammatory cell infiltration, and vascular congestion (**Belsare & Mushrif, 2012**).

9. Statistical analysis

The results are expressed as the mean (M) of individual values $\pm$ standard error of the mean (SEM), calculated using Excel. Comparisons among multiple groups were performed using analysis of variance (ANOVA), considering intoxication status (control vs. intoxicated) and/or treatment factors (extracts and intoxication), followed, when appropriate, by the Student–Newman–Keuls post hoc test. Statistical significance was defined as follows: * $p \leq 0.05$ indicates a significant difference, ** $p < 0.01$ indicates a very significant difference, and *** $p < 0.001$ indicates a highly significant difference compared with the control group. Statistical analyses were performed using SigmaStat software.

Chapter IV:
results and discussion

1. Biochemical assays for the evaluation of liver function

1.1. TGO and TGP

The figures below illustrate the TGO and TGP concentrations in the control group, intoxicated group, groups treated with *Anacyclus pyrethrum*, *Cichorium intybus*, and the mixture, as well as the intoxicated groups treated with *A. pyrethrum*, *C. intybus*, and the mixture.

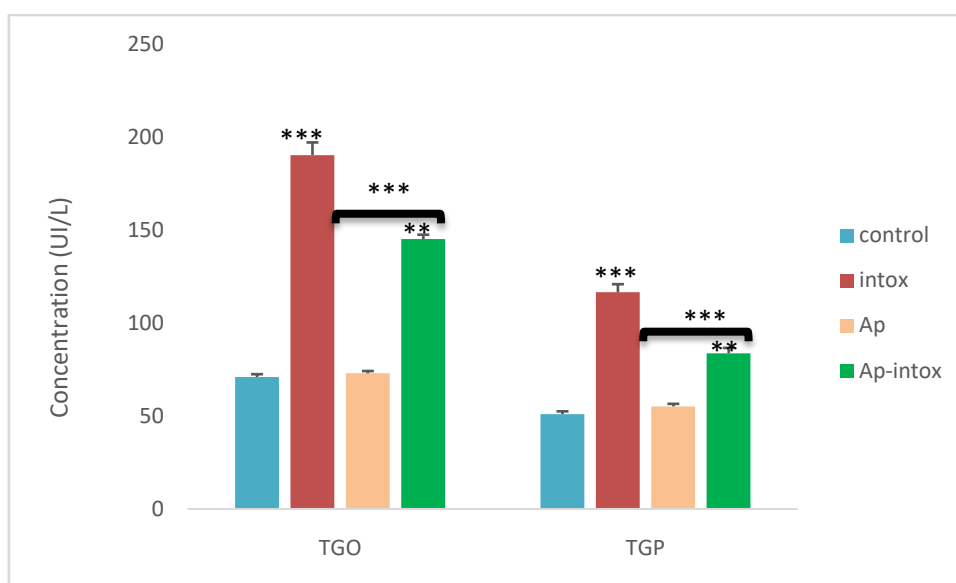


Figure 6: Comparison of TGO and TGP concentrations among control group, intoxicated group, treated control group (*Ap*), and intoxicated treated group (*Ap-intox*). Values are expressed as mean ± SEM (***: $p < 0.001$; **: $p < 0.01$).

The results of TGO and TGP assays showed that the concentrations in the control group were 71 ± 1.56 UI/L and 51 ± 1.56 UI/L, respectively, which were significantly ($p < 0.001$) lower than those observed in the intoxicated group, with values of 190.4 ± 6.8 UI/L and 116.6 ± 4.3 UI/L, respectively. No significant difference was observed between the treated group (73 ± 1.22 UI/L and 55.2 ± 1.36 UI/L, respectively) and the control group ($p \geq 0.05$). In contrast, the intoxicated group treated with *A. pyrethrum* showed TGO and TGP levels of 145.2 ± 2.37 UI/L and 83.8 ± 2.85 UI/L, respectively. These results indicated a highly significant increase compared with the treated control group, while showing a very significant decrease compared with the intoxicated group ($p = 0.002$ and $p = 0.008$, respectively).

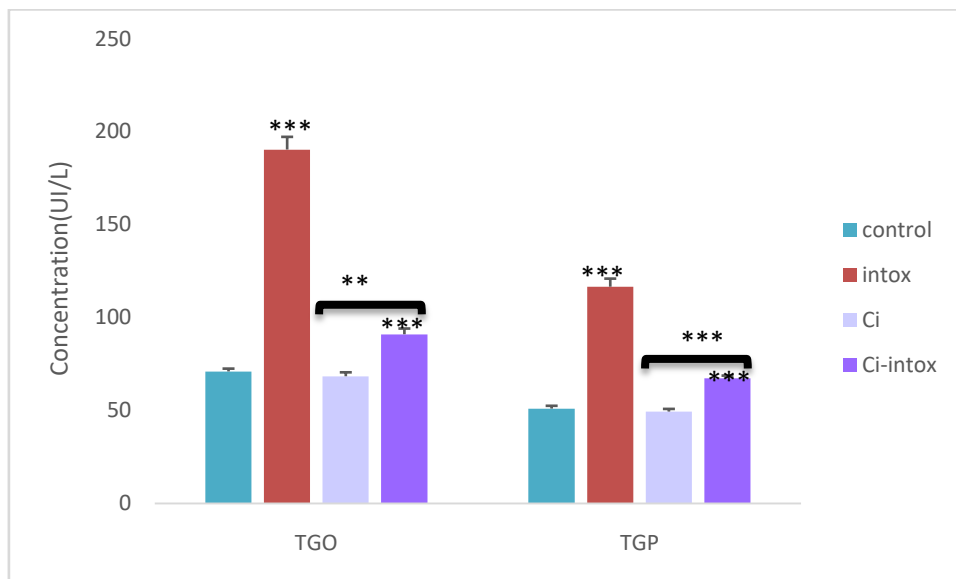


Figure 7: Comparison of TGO and TGP concentrations among control group, intoxicated group, treated control group (*Ci*), and intoxicated treated group (*ci-intox*). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$; **: $p < 0.01$).

The TGO and TGP concentrations in the group treated with *Cichorium intybus* were 68.4 ± 2.13 UI/L and 49.4 ± 1.43 UI/L, respectively, showing no significant difference compared with the control group. The intoxicated group treated with *C. intybus* exhibited TGO and TGP levels of 91.0 ± 3.08 UI/L and 67.4 ± 1.36 UI/L, respectively. These results indicated a significant increase compared with the treated group (TGO: $p = 0.009$; TGP: $p < 0.001$), while showing a highly significant decrease compared with the intoxicated group ($p < 0.001$).

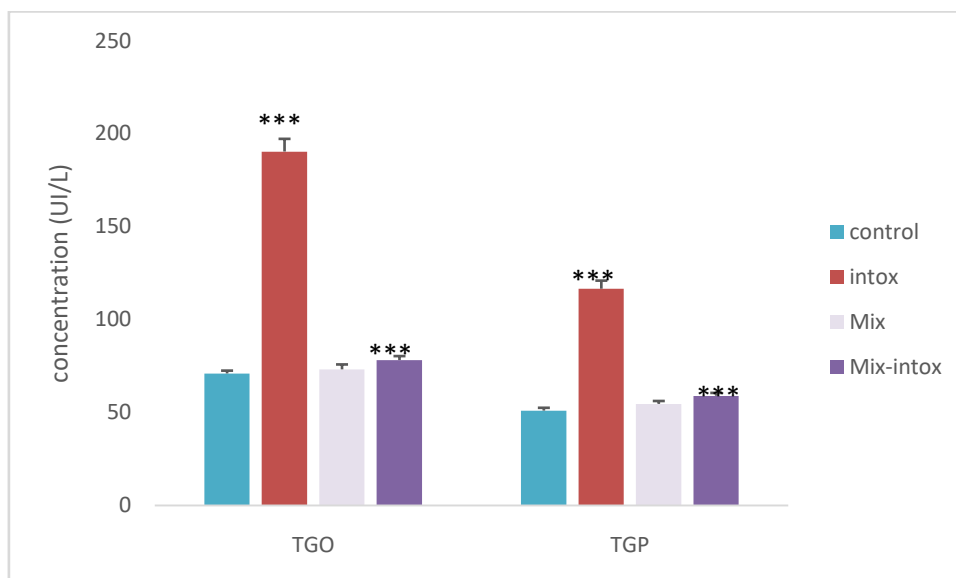


Figure 8: Comparison of TGO and TGP concentrations among control group, intoxicated group, treated control group (mix), and intoxicated treated group (mix-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

In the group treated with the mixture, TGO and TGP levels were 73.2 ± 2.63 UI/L and 54.6 ± 1.57 UI/L, respectively. In the intoxicated group treated with the mixture, TGO and TGP levels were 78.2 ± 2.13 UI/L and 59.0 ± 1.52 UI/L, respectively.

The intoxicated group treated with the mixture showed a highly significant decrease compared with the intoxicated group ($p < 0.001$). Moreover, no significant difference was observed between the mixture-treated group and the mixture-treated intoxicated group.

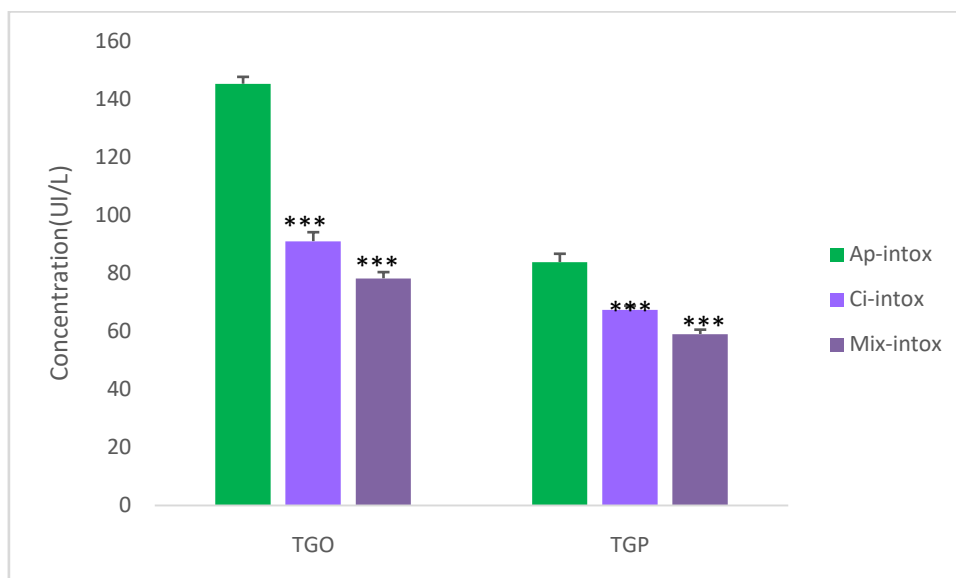


Figure 9: Comparison of TGO and TGP concentrations among, treatedintoxicated group (*Ap*-intox), treated intoxicated group (*ci*-intox) and treated intoxicated group (*mix*-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

Statistical analysis revealed that TGO and TGP levels in the intoxicated group treated with *A. pyrethrum* were significantly higher than those observed in the intoxicated groups treated with *C. intybus* and the mixture ($p < 0.001$). However, no significant difference was detected between the *Ci*-intoxicated group and the *mixt*-intoxicated group.

According to these results, acute Paracetamol intoxication induced a marked increase in TGO and TGP levels. Preventive treatment with *A.pyrethrum*, *C. intybus*, or their mixture significantly reduced TGO and TGP concentrations. Furthermore, *C. intybus* and the mixture exhibited better hepatoprotective effects compared with *A. pyrethrum*.

1.2 bilirubin assay

Figures N, 10,11 and 12 illustrate the concentrations of total and direct bilirubin (mg/dL) across the different experimental groups.

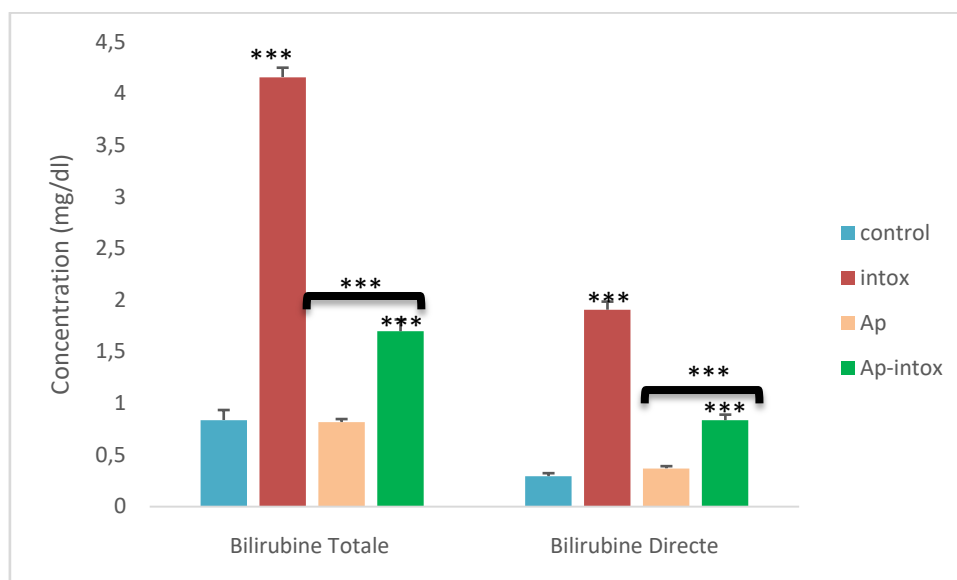


Figure 10: Comparison of Total and Direct Bilirubin Concentrations among control group, intoxicated group, treated control group (*Ap*), and intoxicated treated group (*Ap-intox*). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

The results of total and direct bilirubin assays showed that the concentrations in the control group were $0,838 \pm 0,096$ mg/dl and $0,294 \pm 0,029$ mg/dl, respectively, which were significantly ($p < 0.001$) lower than those observed in the intoxicated group, with values of $4,16 \pm 0,09$ mg/dl and $1,906 \pm 0,078$ mg/dl, respectively. No significant difference was observed between the treated control group ($0,818 \pm 0,03$ mg/dl and $0,37 \pm 0,02$ mg/dl, respectively) and the control group ($p \geq 0.05$). In contrast, the intoxicated group treated with *Anacyclus pyrethrum* showed total and direct bilirubin levels of $1,7 \pm 0,11$ mg/dl and $0,84 \pm 0,054$ mg/dl, respectively. These results indicated a highly significant ($p < 0.001$) increase compared with the treated control group, while showing a very significant decrease compared with the intoxicated group ($p = 0.002$ and $p = 0.008$, respectively).

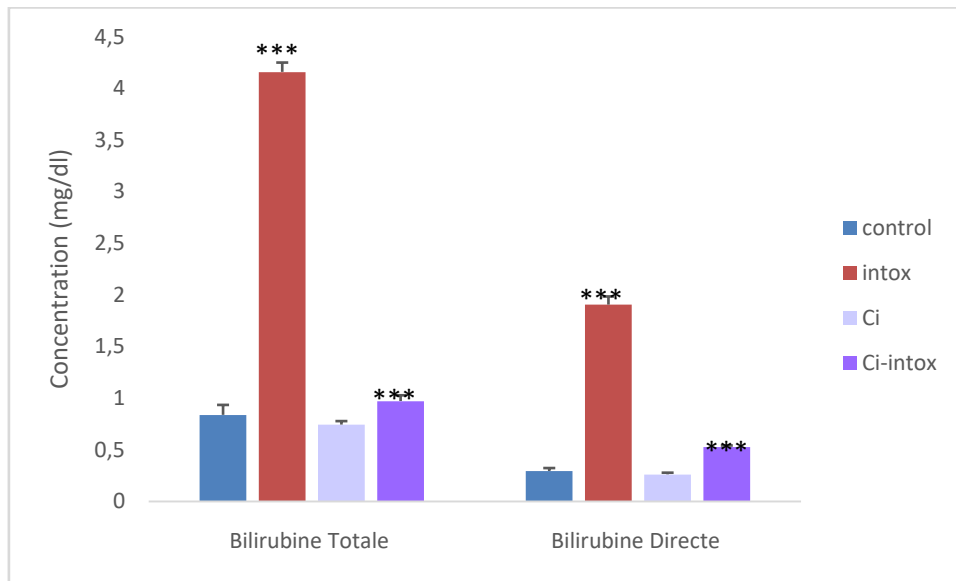


Figure 11: Comparison of Total and Direct Bilirubin Concentrations among control group, intoxicated group, treated control group (Ci), and intoxicated treated group (ci-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

The total and direct bilirubin concentrations in the group treated with *Cichorium intybus* were $0,744 \pm 0,033$ mg/dl and $0,262 \pm 0,016$ mg/dl, respectively, showing no significant difference compared with the control group. The intoxicated group treated with *C. intybus* exhibited total and direct bilirubin levels of $0,97 \pm 0,05$ mg/dl and $0,53 \pm 0,013$ mg/d, respectively. These results indicated the same value compared with the treated group ($p \geq 0,05$), However, it remained significantly ($p < 0.001$) lower compared with the intoxicated group.

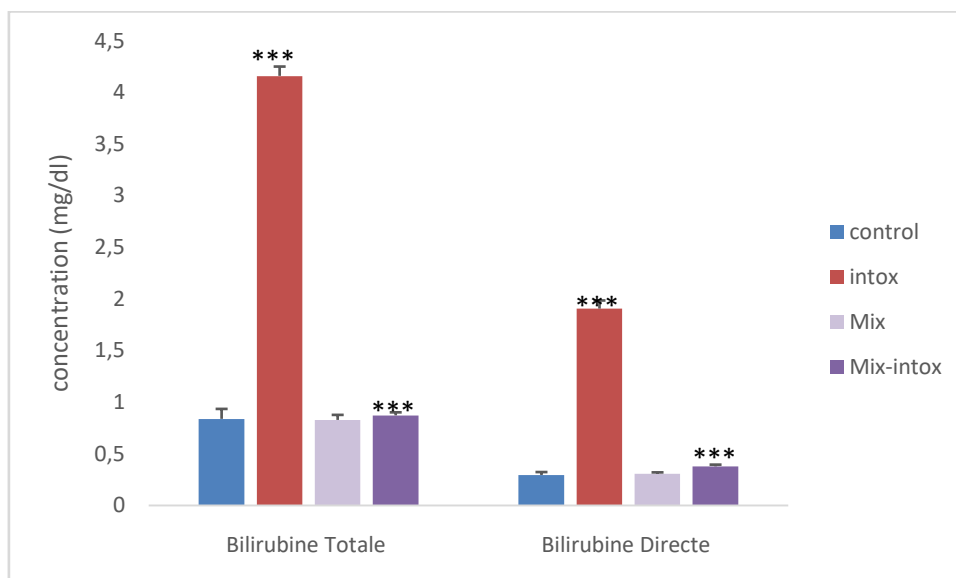


Figure 12: Comparison of Total and Direct Bilirubin Concentrations among control group, intoxicated group, treated control group (mix), and intoxicated treated group (mix-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

In the group treated with the mixture, total and direct bilirubin levels were 0.828 ± 0.048 mg/dl and 0.306 ± 0.014 mg/dl, respectively. In the intoxicated group treated with the mixture, total and direct bilirubin levels were 78.2 ± 2.13 mg/dl and 59.0 ± 1.52 mg/dl, respectively. The intoxicated group treated with the mixture showed a highly significant decrease compared with the intoxicated group ($p < 0.001$). Moreover, no significant difference was observed between the mixture-treated group and the mixture-treated intoxicated group.

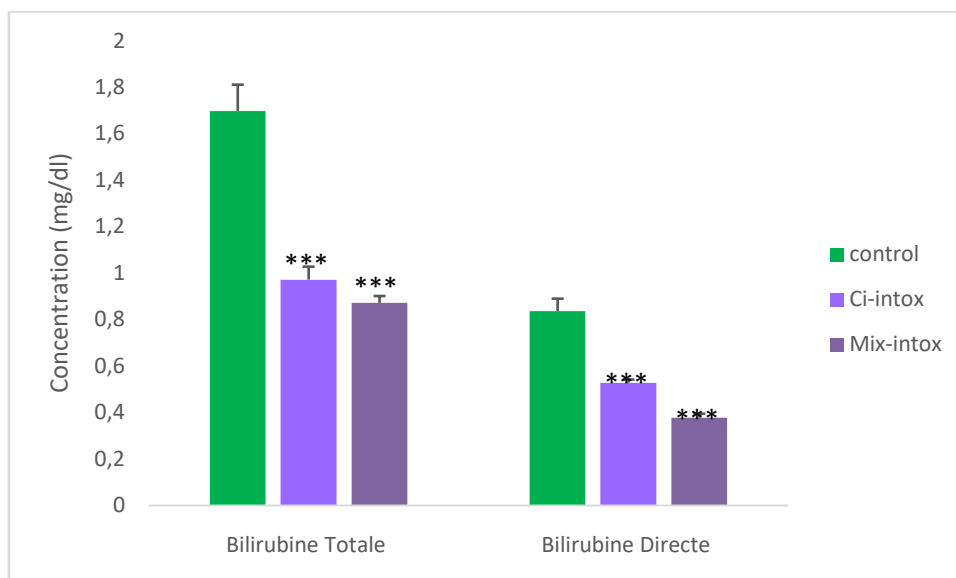


Figure 13: Comparison of Total and Direct Bilirubin Concentrations among, treated intoxicated group (*Ap*-intox), treated intoxicated group (*ci*-intox) and treated intoxicated group (mix-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

Statistical analysis revealed that total and direct bilirubin levels in the intoxicated group treated with *A. pyrethrum* were significantly higher than those observed in the intoxicated groups treated with *C. intybus* and the mixture ($p < 0.001$). However, no significant difference was detected between the Ci-intoxicated group and the mixt-intoxicated group.

According to these results, acute Paracetamol intoxication induced a marked increase in total and direct bilirubin levels. Preventive treatment with *A.pyrethrum*, *C. intybus*, or their mixture significantly reduced total and direct bilirubin concentrations. Furthermore, *C. intybus* and the mixture exhibited better hepatoprotective effects compared with *A. pyrethrum*.

1.3 Gamma-glutamyl transpeptidase assay

The results shows the effect of intoxication and different treatments on the concentration of Gamma-glutamyl transpeptidase (GGT), an important liver enzyme used as a biomarker of hepatic injury. Elevated GGT levels generally indicate liver damage and oxidative stress.

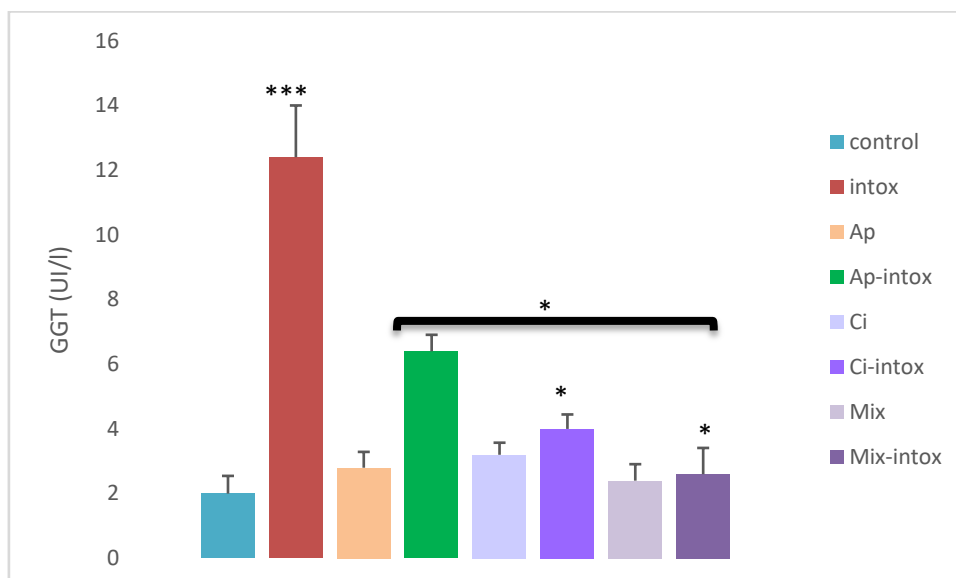


Figure 14: Comparison of Gamma-glutamyl transpeptidase Concentrations among, control group, intoxicated group, treated control groups (*Ap*, *Ci* and *Mix*) treated intoxicated group (*Ap-intox*), (*ci-intox*) and (*mix-intox*). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$; *: $p < 0.05$).

The intoxicated group showed a marked and highly significant ($p < 0.001$) increase in GGT concentration (12.4 ± 1.6 UI/L) compared with the control group (2 ± 0.55 UI/L). This important elevation confirms that intoxication induced severe hepatic damage and altered liver function.

In contrast, the control groups treated with *AP*, *Ci*, and the mixture presented GGT values close to the normal control group (2.8 ± 0.48 , 3.2 ± 0.37 , and 2.4 ± 0.5 UI/L, respectively), with no significant differences ($p \geq 0.05$). These results indicate that the treatments alone did not produce toxic effects on the liver and may be considered safe under normal conditions.

Treatment of intoxicated animals with *AP*, *Ci*, and the mixture reduced GGT concentrations to 6.4 ± 0.5 , 4 ± 0.44 , and 2.6 ± 0.8 UI/L, respectively. Although the *AP*-intoxicated group did not show a significant reduction compared with the intoxicated group ($p \geq 0.05$), the *Ci*-intoxicated and *Mix*-intoxicated groups showed significant decreases ($p = 0.029$ and $p = 0.028$, respectively). This suggests that *Ci* and especially the mixture exhibited a protective effect against intoxication-induced liver damage.

Moreover, the *Mix*-intoxicated group showed GGT values very close to the normal control level, suggesting a near-complete restoration of hepatic function. The significant difference

observed between *Ap*-intoxicated and Mix-intoxicated groups ($p = 0.023$) further supports the superior hepatoprotective activity of the combined treatment.

Overall, these findings demonstrate that intoxication significantly increased GGT activity, while treatment with *Ci* and particularly the mixture effectively attenuated liver injury and restored enzyme levels toward normal values.

1.4 Alkaline phosphatase assay

The figure shows the effect of intoxication and different treatments (*Ap*, *Ci*, and Mix) on alkaline phosphatase (ALP/PAL) concentrations.

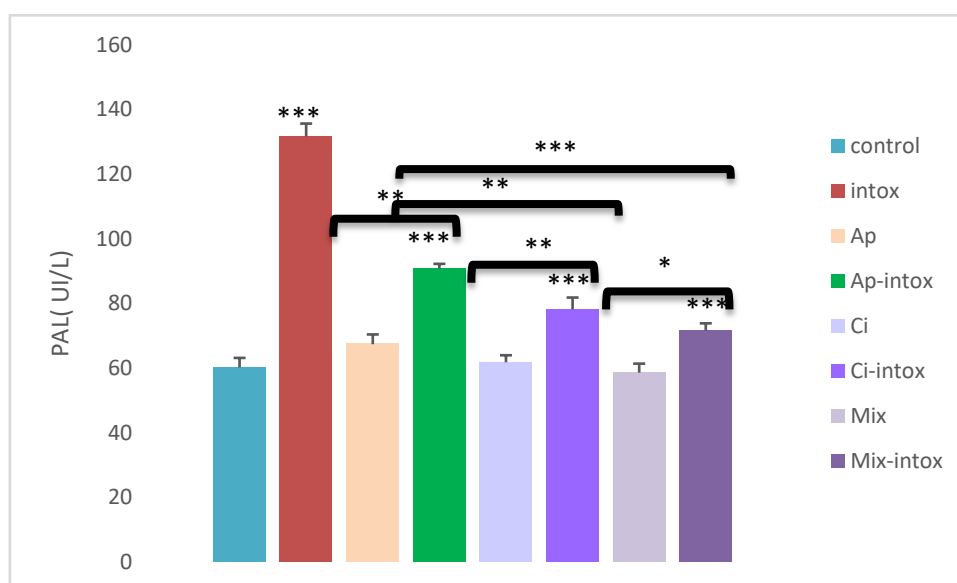


Figure 15: Comparison of alkaline phosphatase concentrations among, control group, intoxicated group, treated control groups (*Ap*, *Ci* and mix) treated intoxicated group (*Ap*-intox), (*ci*-intox) and (*mix*-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).

The intoxicated group presented a marked and highly significant increase in ALP activity (131.6 ± 4 UI/L) compared with the control group (60.2 ± 2.95 UI/L) ($p < 0.001$). This elevation indicates severe hepatic damage and cholestatic alteration induced by intoxication, since ALP is considered an important biochemical marker of liver dysfunction.

In contrast, the control groups treated with *Ap*, *Ci*, and Mix alone showed ALP concentrations close to the normal control values (67.4 ± 3 , 61.8 ± 2.17 , and 58.6 ± 2.78

UI/L, respectively), with no significant difference compared to the control group ($p \geq 0.05$). These results suggest that the treatments themselves did not induce hepatic toxicity.

Treatment of intoxicated animals with *Ap*, *Ci*, and Mix significantly reduced ALP concentrations compared with the intoxicated group ($p < 0.001$ for all comparisons). The *Ap*-intoxicated group showed an ALP value of 90.8 ± 1.42 UI/L, while the *Ci*-intoxicated and Mix-intoxicated groups exhibited lower values of 78.2 ± 3.61 UI/L and 71.6 ± 2.25 UI/L, respectively. However, ANOVA analysis showed that the intoxicated treated groups were significantly higher compared with the corresponding treated control groups. Specifically, significant differences were observed for Control *AP* vs *AP*-intox ($p = 0.002$), Control *Ci* vs *Ci*-intox ($p = 0.005$), and Control Mix vs Mix-intox ($p = 0.015$).

Moreover, comparison among treated intoxicated groups revealed that the Mix treatment produced the greatest improvement, followed by *Ci* and then *Ap*. Indeed, ALP levels were significantly Higher in the *Ap*-intoxicated group compared with the *Ci*-intoxicated group and Mix-intoxicated group ($p = 0.004$, $p < 0.001$ respectively), whereas the difference between *Ci*-intoxicated and Mix-intoxicated groups was not statistically significant ($p \geq 0.05$). These findings indicate that the combined treatment may exert a stronger hepatoprotective effect, probably through synergistic antioxidant and anti-inflammatory activities. Overall, the results indicate that intoxication caused a pronounced increase in ALP concentration, reflecting liver damage, whereas treatment with *Ap*, *Ci*, and especially the Mix formulation effectively attenuated these biochemical alterations and improved liver function.

1.5 total protein assay

The figure below presents the total protein concentration (g/L) in the different experimental groups. Reduced protein levels may indicate impaired liver function, as alterations in protein synthesis are often associated with hepatic dysfunction.

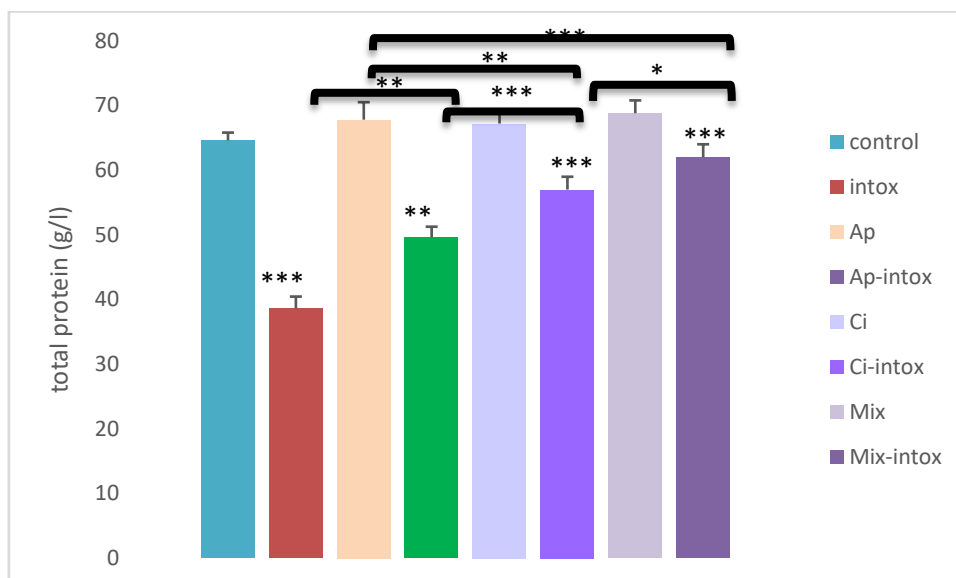


Figure 16: Comparison of total protein concentrations among, control group, intoxicated group, treated control groups (*Ap*, *Ci* and *Mix*) treated intoxicated group (*Ap-intox*), (*Ci-intox*) and (*Mix-intox*). Values are expressed as mean ± SEM (***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$).

The results show that total protein concentration was markedly affected by intoxication and improved after treatment with *Ap*, *Ci*, and especially the mixed treatment. In the control group, the total protein level was normal (64.6 ± 1.2 g/L), whereas the intoxicated group showed a significant ($p < 0.001$) decrease to 38.6 ± 1.86 g/L. This strong reduction indicates that intoxication caused severe impairment in protein metabolism and liver synthetic function, leading to hypoproteinemia. The control groups treated with *Ap*, *Ci*, and *Mix* alone showed protein concentrations of 67.8 ± 2.7 g/L, 67.2 ± 1.56 g/L, and 68.8 ± 1.98 g/L, respectively. Since there was no significant difference compared with the normal control group ($p \geq 0.05$), these treatments did not produce toxic effects and maintained normal protein synthesis.

Among intoxicated animals receiving treatments, the *Ap*-intoxicated group showed a partial preventive of total protein concentration (49.6 ± 1.66 g/L), significantly higher than the intoxicated group ($p = 0.002$), but still significantly lower than the corresponding control *AP* group ($p = 0.008$). This suggests that *Ap* treatment provided moderate protective activity against intoxication-induced protein depletion. The *Ci*-intoxicated group presented a greater improvement, with total protein levels reaching 57 ± 1.61 g/L. This increase was highly significant compared with the intoxicated group ($p < 0.001$), although

levels remained significantly lower than the control *Ci* group ($p < 0.001$). These results indicate that *Ci* had a stronger restorative effect than *Ap*.

The Mix-intoxicated group showed the highest preventive, with total protein concentration reaching 62 ± 2 g/L, very close to the normal control value. The mixed treatment significantly improved protein levels compared with the intoxicated group ($p < 0.001$). Although a slight difference remained compared with the control Mix group ($p = 0.012$), the combined treatment demonstrated the best protective effect against intoxication.

Furthermore, comparison between treated intoxicated groups revealed that *Ci*-intoxicated animals had significantly higher protein levels than *Ap*-intoxicated animals ($p = 0.008$), while the Mix-intoxicated group showed even greater improvement compared with *Ap*-intoxicated animals ($p < 0.001$). No significant difference was observed between *Ci*-intoxicated and Mix-intoxicated groups ($p \geq 0.05$), suggesting that both treatments were highly effective, with the mixed treatment producing near-normal restoration. Overall, the results demonstrate that intoxication significantly reduced total protein concentration, whereas treatment with *Ap*, *Ci*, and particularly the mixed treatment attenuated this effect and improved protein synthesis capacity.

The biochemical findings of the present study demonstrated that acute intoxication with paracetamol induced marked alterations in hepatic function biomarkers, characterized by significant elevations in serum transaminases, including glutamate oxaloacetate transaminase (AST/TGO) and glutamate pyruvate transaminase (ALT/TGP), as well as increased levels of total and direct bilirubin, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP). Conversely, total protein concentrations were significantly reduced following intoxication. These findings are in agreement with previous investigations demonstrating the hepatotoxic potential of paracetamol overdose and its deleterious effects on liver biochemical parameters. For instance, **Henneh et al. (2022)** reported that oral administration of paracetamol at a dose of 3000 mg/kg induced significant increases ($p < 0.01$) in serum ALT, AST, ALP activities, and total bilirubin levels, accompanied by a significant reduction ($p < 0.05$) in total protein concentrations compared with control animals. Similarly, **Alkufaishi and Rashid (2025)** demonstrated that repeated oral administration of paracetamol (500 mg/kg for 14 days) resulted in

marked increases in ALT (245.6 ± 32.7 U/L), AST (280.4 ± 40.5 U/L), ALP (320.8 ± 35.9 U/L), and bilirubin levels (2.8 ± 0.6 mg/dL), reflecting substantial hepatic dysfunction.

Comparable observations were reported by **Alhusaini et al. (2022)**, where acute liver injury induced by a single oral dose of paracetamol (1000 mg/kg) caused significant elevations in serum AST and ALT levels, indicating extensive hepatocellular membrane disruption and leakage of intracellular enzymes into the circulation. **Likewise, Teixeira et al. (2025)** demonstrated that administration of a single toxic dose of paracetamol (3 g/kg) significantly increased serum ALT, AST, and ALP activities ($p < 0.001$), further confirming the susceptibility of hepatic tissue to paracetamol-induced injury. Furthermore, **Feki et al. (2022)** showed that administration of paracetamol at 2 g/kg significantly increased plasma levels of AST, ALT, GGT and ALP with increases of 47.2%, 113.9%, 337.5%, and 196.4%, respectively compared to the control group. The marked elevation in GGT activity was attributed to hepatocellular and canalicular membrane damage, given that this enzyme is predominantly localized within the canalicular domain of hepatocyte plasma membranes under physiological conditions. Additionally, increased ALP activity was associated with cholestatic disturbances and impaired biliary function following paracetamol intoxication.

The present study demonstrated that treatment with *Cichorium intybus*, *Anacyclus pyrethrum*, and their combination significantly ameliorated paracetamol-induced hepatic dysfunction, as evidenced by reductions in serum hepatic biomarkers, including transaminases and cholestatic enzymes, accompanied by restoration of total protein levels. These findings indicate a pronounced hepatoprotective potential of both plant extracts, either individually or in combination, likely through preservation of hepatocellular integrity and improvement of hepatic synthetic function.

The hepatoprotective properties of *C. intybus* observed in the present work are supported by several experimental studies. **Nallamilli et al. (2013)** reported that administration of an alcoholic extract of *C. intybus* (400 mg/kg, p.o.) significantly attenuated carbon tetrachloride-induced hepatic injury by reducing elevated serum SGOT, SGPT, ALP, and total bilirubin levels. Animals intoxicated with carbon tetrachloride (1 mL/kg) exhibited marked increases in SGOT, SGPT, SALP, and bilirubin values, whereas treatment with *C. intybus* (15.5 ± 0.72 IU/L, 59.23 ± 0.55 IU/L, 2840 ± 40.41 IU/L and

7.45±0.25 mg/dl respectively) significantly normalized these parameters. Similarly, **Riaz et al. (2024)** investigated the hepatoprotective potential of *Cichorium intybus* against paracetamol-induced hepatotoxicity in rats. Hepatic injury was induced by oral administration of paracetamol for 5 days, while the treatment group received *C. intybus* extract (200 mg) dissolved in water for 10 days. Biochemical evaluation demonstrated that paracetamol intoxication significantly disrupted liver function parameters, as evidenced by elevated serum transaminase activities. Treatment with *C. intybus* attenuated these alterations by reducing ALT and AST levels, regarding alkaline phosphatase activity, paracetamol administration caused a marked reduction in ALP levels compared with the control group, reflecting hepatic dysfunction. However, treatment with *C. intybus* restored ALP activity toward normal values, total bilirubin concentrations remained relatively stable between experimental groups, suggesting limited effects on bilirubin metabolism under these conditions. Moreover, administration of *C. intybus* significantly improved total protein concentrations ($p \leq 0.05$), approaching normal control values, thereby indicating restoration of hepatic synthetic capacity. The observed biochemical improvements collectively demonstrate that *C. intybus* exerts protective effects against paracetamol-induced liver injury.

Comparable findings were reported by **Khalid et al. (2018)**, where preventive treatment with *C. intybus* (100 mg/kg) significantly reduced serum SGOT, SGPT, ALP, and direct bilirubin levels in carbon tetrachloride-intoxicated rats. Likewise, **Elgengaihi et al. (2016)** demonstrated that oral administration of *C. intybus* (500 mg/kg) significantly reduced ALT, AST, ALP, and GGT activities while simultaneously improving total protein concentrations and decreasing bilirubin levels in animals receiving repeated carbon tetrachloride exposure. **Li et al. (2014)** further confirmed these observations using a chronic carbon tetrachloride hepatotoxicity model. Administration of *Cichorium intybus* extract significantly reduced AST and ALT levels from 711.14 ± 165.78 U/L and 738.81 ± 231.66 U/L in intoxicated animals to 194.86 ± 44.25 U/L and 123.65 ± 29.93 U/L, respectively ($p < 0.01$), indicating substantial protection against hepatocellular damage.

Additional evidence was provided by **Saggu et al. (2014)**, who demonstrated that *chicory* fruit extract (100 mg/kg) significantly reduced elevations in ALT, AST, ALP, GGT, and total bilirubin induced by 4-tert-octylphenol toxicity.

Furthermore, strong evidence supporting the efficacy of *C. intybus* against paracetamol toxicity was reported by **Al-Snai et al. (2019)**. Pretreatment with aqueous-methanolic seed extract significantly reduced acetaminophen-induced mortality and normalized hepatic enzymes. In rats receiving paracetamol (640 mg/kg), ALP, GOT, and GPT levels increased markedly to 393 ± 28 , 767 ± 215 , and 692 ± 191 IU/L, respectively, whereas pretreatment with *C. intybus* reduced these values to 228 ± 16 , 68 ± 10 , and 41 ± 8 IU/L ($p < 0.01$).

The hepatoprotective effect of *A. pyrethrum* observed in the present investigation is also consistent with previous reports. **Usmani et al. (2016)** evaluated the protective effect of *A. pyrethrum* extract against hepatotoxicity induced by isoniazid and rifampicin. Hepatotoxic animals exhibited marked increases in SGPT, SGOT, ALP, bilirubin levels, and decreased total protein concentrations. Treatment with *A. pyrethrum* at 200 and 400 mg/kg significantly attenuated these alterations in a dose-dependent manner. At 400 mg/kg, *A. pyrethrum* significantly reduced SGPT, SGOT, ALP, and bilirubin levels to 107.4 ± 2.0 IU/L, 114.6 ± 1.8 IU/L, 133.66 ± 1.8 IU/L, and 1.17 ± 0.09 mg/dL, respectively, while increasing total protein levels to 4.78 ± 0.18 g/dL ($p < 0.01$). These findings suggest that *A. pyrethrum* exerts substantial hepatoprotective activity through attenuation of hepatocellular damage and restoration of hepatic metabolic function.

Collectively, the reduction in serum transaminases, cholestatic enzymes, and bilirubin levels, together with restoration of total protein concentrations observed after treatment with *C. intybus*, *A. pyrethrum*, and their combination, suggests preservation of hepatocyte membrane integrity and improvement of hepatic functional capacity. The hepatoprotective effects may be attributed to the abundance of bioactive phytochemicals, including phenolic compounds, flavonoids, coumarins, and other antioxidant constituents, which reduce oxidative stress, inhibit lipid peroxidation, stabilize cellular membranes, and promote tissue regeneration.

2. Oxidative stress assay

2.1 Catalase activity:

The figure below illustrates the catalase (CAT) activity, expressed as $\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$ protein, among the different experimental groups

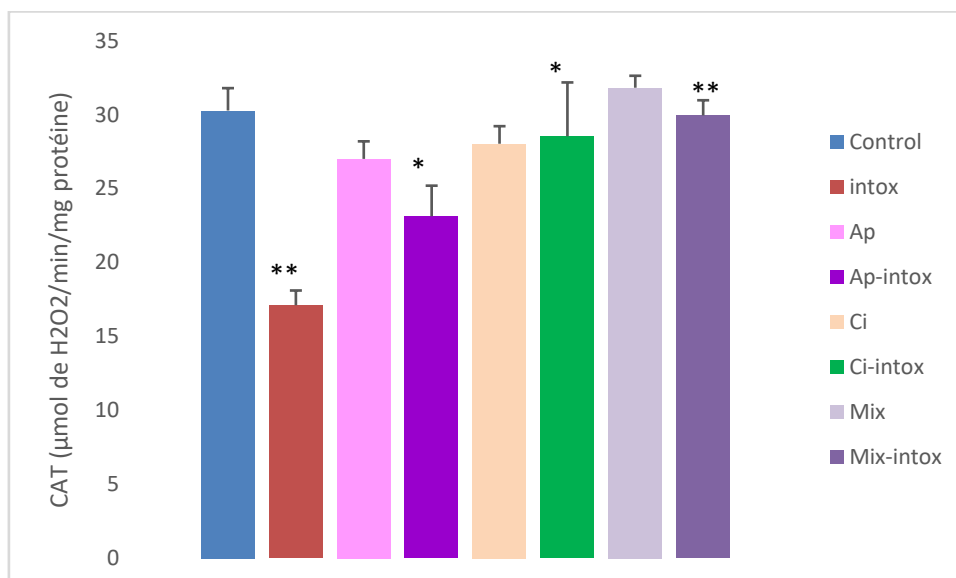


Figure 17: Comparison of catalase activity among, control group, intoxicated group , treated control groups (*Ap*, *Ci* and *Mix*)treatedintoxicated group (*Ap*-intox),(*ci*-intox) and (*mix*-intox). Values are expressed as mean ± SEM (**: $p < 0.01$;*: $p < 0.05$).

The CAT activity in the control group was 30.28 ± 1.51 $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ protein, whereas in the intoxicated group it was 17.11 ± 0.83 $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ protein. According to the ANOVA statistical analysis, a highly significant decrease ($p = 0.007$) was observed in the intoxicated group compared with the control group. The CAT activity in the control groups treated with *Ap*, *Ci*, and *Mix* was 27.01 ± 0.54 , 28.04 ± 1.19 , and 31.82 ± 0.81 $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ protein, respectively. No significant differences were observed compared with the control group.

In the groups pretreated with *Ap*, *Ci*, and *Mix* before intoxication, the catalase activity was 23.13 ± 2.07 , 28.54 ± 3.64 , and 29.95 ± 1.03 $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ protein, respectively. These values were significantly higher compared with the intoxicated group, with $p = 0.045$, $p = 0.024$, and $p = 0.001$, respectively. No significant differences were observed among the treated intoxicated groups.

2.2 The malondialdehyde concentration

Figure N illustrates the concentration of MDA (nmol/mg protein) in the control, intoxicated, control-treated, and preventive-treated intoxicated groups.

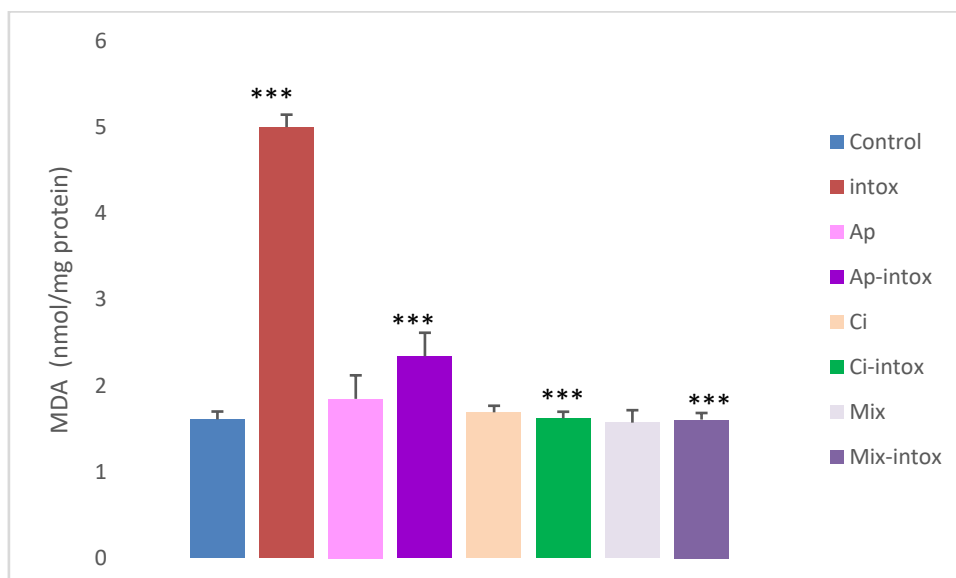


Figure 18: Comparison of The malondialdehyde concentration among, control group, intoxicated group , treated control groups (*Ap*, *Ci* and *Mix*)treatedintoxicated group (*Ap*-intox),(*ci*-intox) and (*mix*-intox). Values are expressed as mean $\pm$ SEM (***: $p < 0.001$).

The figure demonstrates that intoxication significantly increased MDA levels, reflecting enhanced oxidative stress and lipid peroxidation. The intoxicated group exhibited a marked elevation in MDA concentration (4.99 ± 0.19 nmol/mg protein) compared with the control group (1.61 ± 0.10 nmol/mg protein) ($p < 0.001$). Control groups treated with *Ap*, *Ci*, and *Mix* alone showed MDA concentrations of 1.85 ± 0.18 , 1.69 ± 0.09 , and 1.57 ± 0.14 nmol/mg protein, respectively, with no significant differences compared with the normal control group ($p \geq 0.05$), indicating that these treatments did not induce oxidative damage.

Administration of *Ap*, *Ci*, and *Mix* to intoxicated animals significantly reduced MDA concentrations compared with the intoxicated group ($p < 0.001$). The *Ap*-treated intoxicated group showed a partial reduction in MDA levels (2.34 ± 0.27 nmol/mg protein), whereas the *Ci*-treated intoxicated group (1.62 ± 0.07 nmol/mg protein) and the *Mix*-treated intoxicated group (1.60 ± 0.07 nmol/mg protein) restored MDA concentrations to values close to those of the control group.

Furthermore, no significant differences were observed between treated control groups and their corresponding intoxicated treated groups ($p \geq 0.05$), suggesting a substantial protective effect against oxidative stress. Likewise, the absence of significant

differences among the *Ap*-intoxicated, *Ci*-intoxicated, and Mix-intoxicated groups indicates that all treatments reduced lipid peroxidation, although *Ci* and Mix exhibited MDA values closest to normal control levels.

Paracetamol is a widely used analgesic and antipyretic drug; however, overdose can induce severe hepatotoxicity and represents a major cause of acute liver failure. A portion of paracetamol undergoes metabolism by cytochrome P450 enzymes to generate the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which is normally detoxified through conjugation with glutathione (GSH). During overdose conditions, depletion of GSH enhances oxidative stress, promotes reactive oxygen species generation, induces lipid peroxidation and mitochondrial dysfunction, and ultimately leads to hepatocellular injury and liver necrosis (**Zakaria et al., 2018; Tittarelli et al., 2017**). The liver counteracts oxidative damage through endogenous antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), which are essential for maintaining redox homeostasis and cellular protection (**Teixeira et al., 2025**).

CAT is a major enzymatic antioxidant involved in cellular defense against oxidative stress through the detoxification of ROS, particularly hydrogen peroxide and superoxide-derived metabolites. This enzyme is widely distributed in animal tissues, including the liver and erythrocytes, where it plays a critical role in preventing oxidative damage by limiting the formation of highly reactive hydroxyl radicals (**Henneh et al., 2022**). In the present study, paracetamol intoxication significantly reduced CAT activity, indicating impairment of the endogenous antioxidant defense system. Conversely, treatment with *Cichorium intybus*, *Anacyclus pyrethrum*, and their combination significantly restored CAT activity compared with the intoxicated group, suggesting an enhancement of antioxidant capacity and protection against oxidative damage.

MDA is a major secondary product of lipid peroxidation, is commonly used as a sensitive biomarker of oxidative stress and membrane damage. Elevated MDA levels reflect enhanced free radical-mediated degradation of membrane lipids and indicate increased cellular injury (**Fauziah et al., 2018**). Our results demonstrated that paracetamol intoxication significantly increased MDA concentrations, whereas administration of both

plant extracts and their combination reduced MDA accumulation, indicating attenuation of lipid peroxidation and oxidative injury.

These findings are supported by previous studies investigating paracetamol-induced oxidative stress. **Henneh et al. (2022)** reported that paracetamol administration significantly decreased CAT activity ($p < 0.05$) compared with control animals, reflecting exhaustion of antioxidant defenses. Similarly, **Alhusaini et al. (2022)** demonstrated that acute liver injury induced by a single oral dose of paracetamol (1000 mg/kg) significantly increased MDA levels ($p < 0.001$), confirming extensive oxidative damage. **Teixeira et al. (2025)** also observed a significant increase in MDA concentrations following administration of a toxic dose of paracetamol (3 g/kg), where MDA levels increased by 169% relative to controls ($p < 0.001$), indicating pronounced lipid peroxidation. Comparable observations were reported by **Feki et al. (2022)**, who showed that paracetamol overdose (2 g/kg) increased hepatic MDA concentrations by 245.2% while simultaneously reducing CAT activity by 56.9%. The authors attributed these alterations to excessive ROS accumulation, which promotes membrane lipid peroxidation and weakens endogenous antioxidant defenses. Since CAT catalyzes the conversion of hydrogen peroxide into water and oxygen, reduced CAT activity contributes to enhanced oxidative stress and hepatocellular injury.

The restoration of oxidative stress markers observed in the present study is consistent with previous reports demonstrating the antioxidant properties of *C. intybus*. **Khalid et al. (2018)** reported that administration of *C. intybus* (100 mg/kg) increased CAT activity in intoxicated animals, indicating improved antioxidant status. Similarly, **Li et al. (2014)** demonstrated that treatment with *Cichorium intybus* extract reduced MDA concentrations in animals exposed to carbon tetrachloride, suggesting attenuation of lipid peroxidation. **Saggu et al. (2014)** further confirmed these findings by showing that administration of hydroalcoholic chicory fruit extract significantly reduced hepatic MDA levels by 67.3% in animals intoxicated with 4-tert-octylphenol ($p < 0.001$). Moreover, chicory treatment significantly increased CAT activity, indicating recovery of antioxidant defense mechanisms. The authors suggested that these protective effects were related to the phytochemical composition of *chicory*, particularly its phenolic constituents and antioxidant compounds.

The antioxidant activity of *A. pyrethrum* observed in this study is also supported by previous literature. **Manouze et al. (2017)** demonstrated that aqueous and methanolic extracts of *A. pyrethrum* roots possess strong antioxidant activity, which was attributed to the presence of bioactive phytochemicals including flavonoids, alkaloids, tannins, terpenoids, and saponins. These compounds are known to scavenge free radicals, inhibit lipid peroxidation, and strengthen endogenous antioxidant defense systems. Overall, the reduction in MDA levels together with restoration of CAT activity following treatment with *C. intybus*, *A. pyrethrum*, and their combination suggests that these plants exert hepatoprotective effects primarily through antioxidant mechanisms involving suppression of oxidative stress, inhibition of lipid peroxidation, and reinforcement of endogenous antioxidant defenses.

Furthermore, the combined treatment may indicate synergistic interactions between phytoconstituents, resulting in enhanced protection against paracetamol-induced oxidative injury.

3. Histological study

A hepatic parenchyma characterized by the Glisson's capsule, which gives rise to connective tissue septa delimiting lobules composed of hepatocytes separated by sinusoidal spaces. The lobules are bordered by portal spaces containing a vein, an artery, and a bile ductule.

In the control group, macroscopic examination showed a liver measuring $4.5 \times 3.5 \times 0.3$ cm, with a normal volume and a homogeneous appearance. Histological analysis revealed a hepatic parenchyma of near-normal aspect, with only mild post-mortem changes observed. In the group treated with *A.pyrethrum* , *C.intybus* and combined treatment macroscopic examination showed a homogeneous liver measuring $4 \times 3.5 \times 0.3$ cm, $4 \times 3.5 \times 0.3$ cm and $4 \times 3.5 \times 0.5$ cm respectively. Microscopic examination showed a normal hepatic parenchyma without evidence of necrosis.

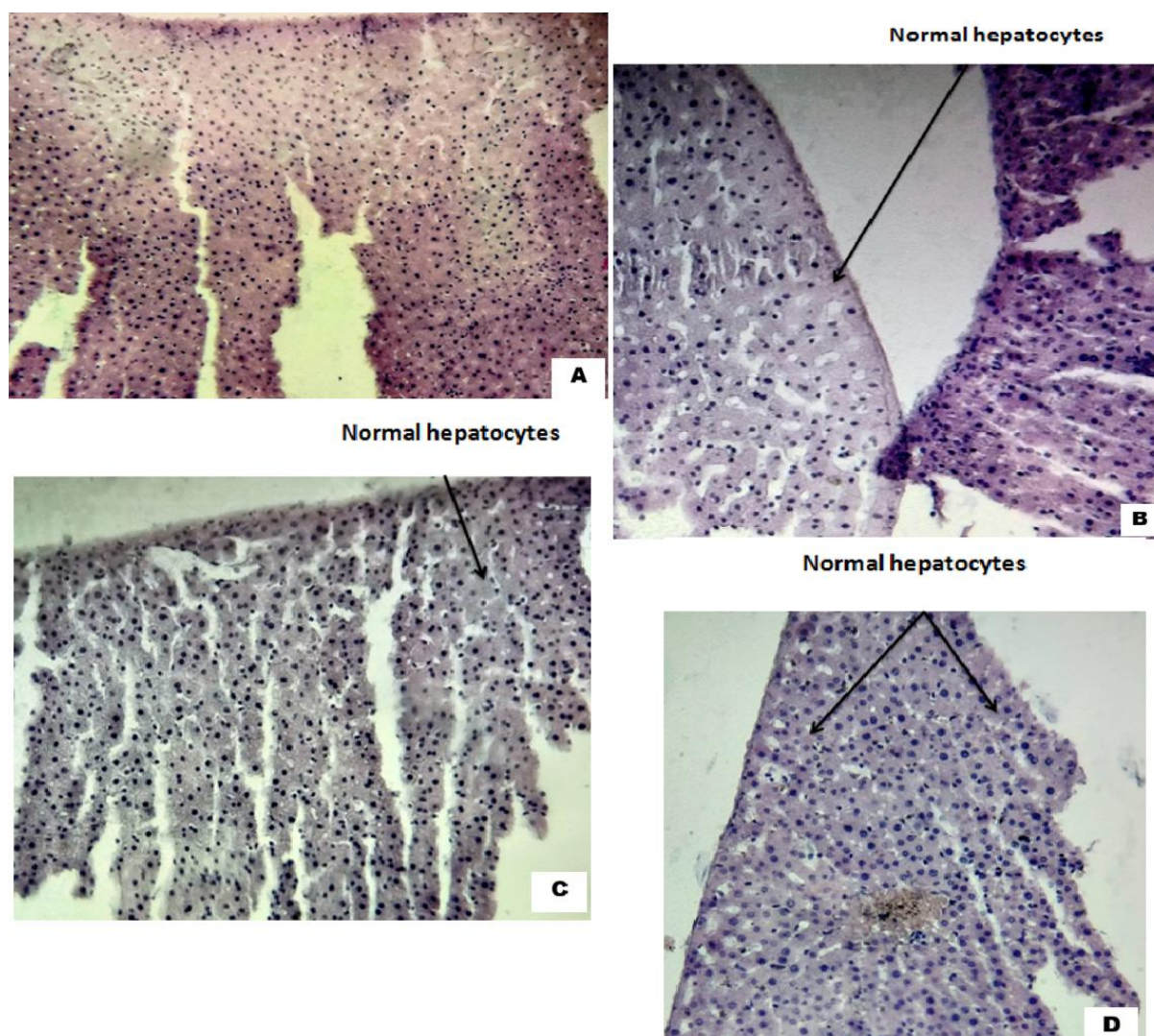


Figure 19: Light microscopy of hepatic tissue showing normal architecture (×40). (A) Control group; (B) control group treated with *A.pyrethrum*; (C) control group treated with *C.intybus*; (D) control group treated with the mixture.

In the Intoxicated group, macroscopic examination revealed an enlarged and heterogeneous liver with dimensions of $5 \times 3 \times 0.5$ cm. Histological analysis showed a hepatic parenchyma characterized by a moderate lobular lymphocytic inflammatory infiltrate, hepatocellular ballooning, and cytoplasmic clarification. The hepatocytes appeared pale, sometimes strongly eosinophilic, with pycnotic nuclei.

In the intoxicated group treated with *Anacyclus pyrethrum*, macroscopic examination showed a homogeneous liver with dimensions of $5 \times 4 \times 0.5$ cm. Histological analysis revealed a hepatic parenchyma characterized by a moderate lobular and centrolobular lymphocytic inflammatory infiltrate. Persistent hepatocellular ballooning and

cytoplasmic clarification were also observed.

In the intoxicated group treated with *Cichorium intybus*, macroscopic examination showed a homogeneous liver measuring $4.5 \times 3.5 \times 0.5$ cm. Histological analysis revealed a hepatic parenchyma characterized by a moderate lobular and centro-lobular lymphocytic inflammatory infiltrate. No hepatocellular ballooning or cytoplasmic clarification was observed, and the hepatocytes appeared of near-normal aspect.

In the intoxicated group treated with the combined treatment, macroscopic examination showed a homogeneous liver measuring $5 \times 4 \times 0.5$ cm. Histological analysis revealed a hepatic parenchyma with a mild lobular lymphocytic inflammatory infiltrate. No hepatocellular ballooning or cytoplasmic clarification was observed, and the hepatocytes appeared of near-normal aspect.

Inflammation is a central mechanism underlying the progression of hepatic injury following exposure to toxic agents. As a major immunological organ, the liver responds to hepatotoxic insults through activation of inflammatory pathways, particularly via Kupffer cells, which release pro-inflammatory mediators in response to hepatocyte necrosis or direct toxic stimulation. These mediators further amplify oxidative stress, inflammatory infiltration, and tissue damage, thereby aggravating liver injury (**Kim et al., 2009**).

In the present study, paracetamol intoxication induced marked histopathological alterations, confirming severe hepatic injury. These observations are consistent with previous studies demonstrating that paracetamol overdose disrupts hepatic architecture and promotes inflammatory and degenerative changes. **Henneh et al. (2022)** reported that rats intoxicated with paracetamol (3000 mg/kg) exhibited single-cell hepatocellular necrosis, ballooning degeneration, and sinusoidal dilatation, indicating early hepatocyte injury and structural disruption. Similarly, **Attaullah et al. (2023)** demonstrated that paracetamol-treated animals developed severe necrosis, fibrosis, steatosis, inflammatory infiltration, dilation of the central vein, and proliferation of Kupffer cells, reflecting extensive hepatic remodeling and inflammation.

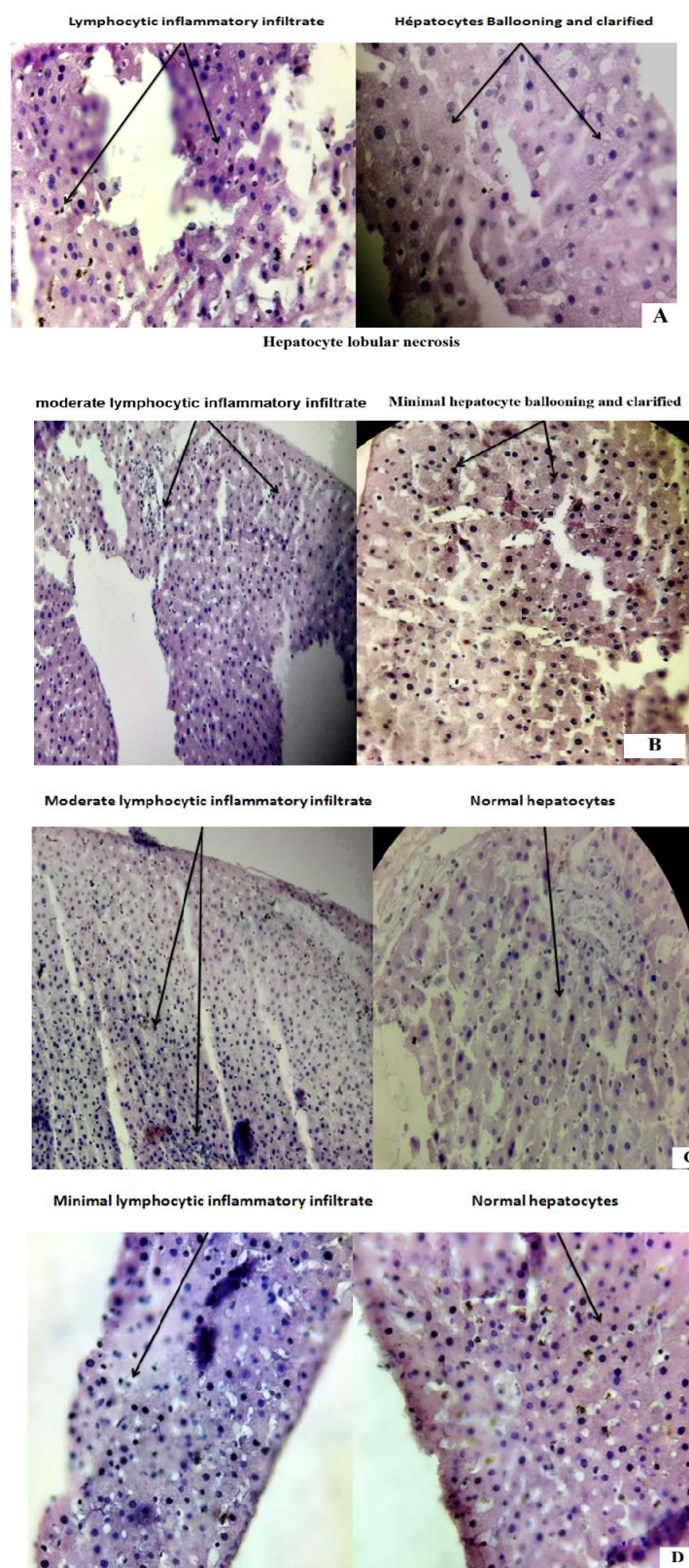


Figure 20: Light microscopy of hepatic tissue (×40). (A) intoxicated group; (B) intoxicated group treated with *A.pyrethrum*; (C) intoxicated group treated with *C. intybus*; (D) intoxicated group treated with the mixture.

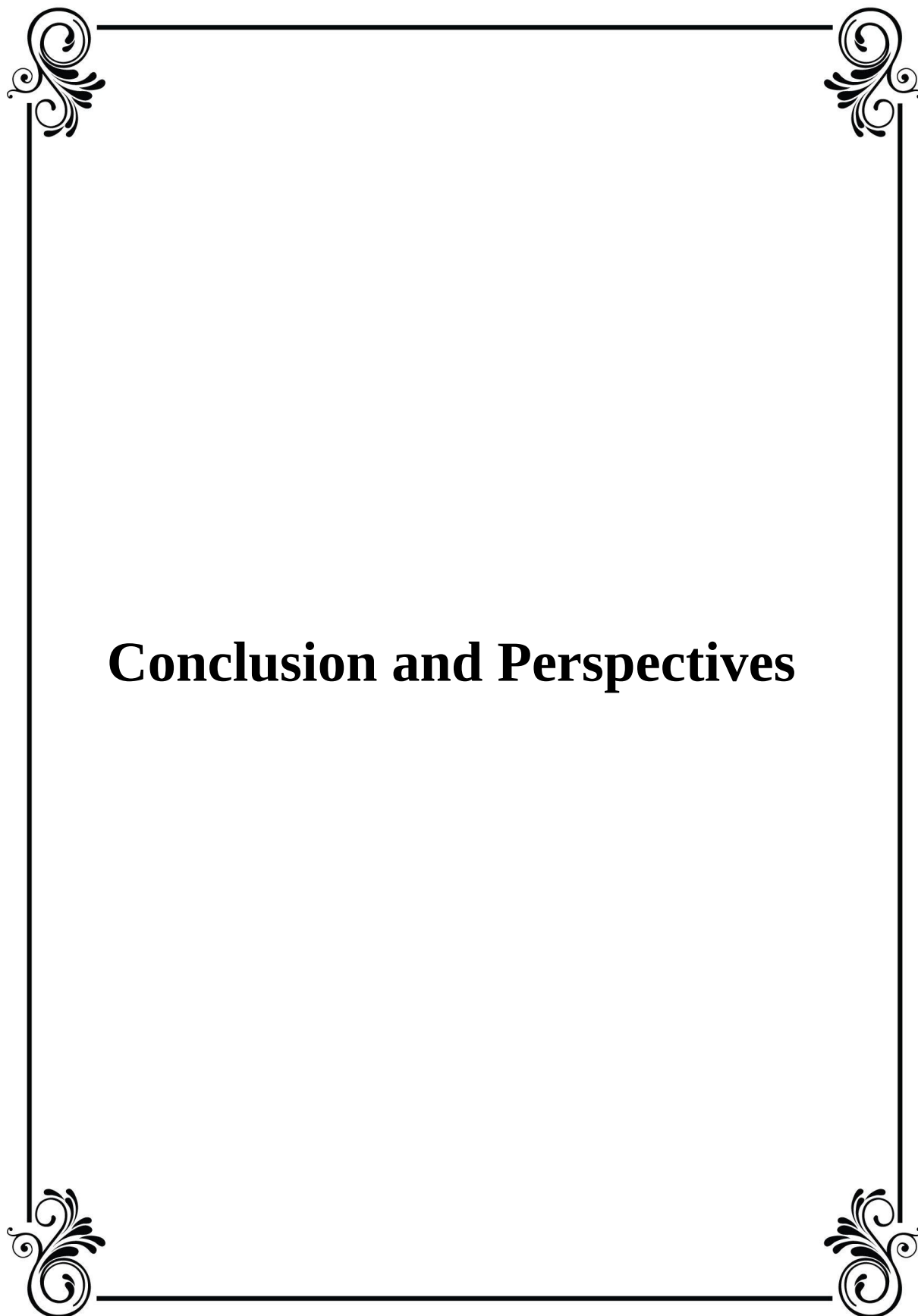
Comparable findings were reported by **Alkufaishi and Rashid (2025)**, who showed that repeated administration of paracetamol induced dose-dependent histopathological lesions characterized by hepatocellular necrosis, sinusoidal congestion, inflammatory cell infiltration, cytoplasmic vacuolization, and fatty degeneration. Likewise, **Alhusaini et al. (2022)** observed congested central veins surrounded by degenerated and vacuolated hepatocytes following acute paracetamol intoxication, while **Teixeira et al. (2025)** demonstrated significant expansion of necrotic areas within hepatic tissue after administration of a toxic dose of paracetamol. **Feki et al. (2022)** further reported profound architectural disruption characterized by leukocyte infiltration, steatosis, fibrosis, hepatocyte vacuolization, confluent necrotic areas, and collagen deposition, confirming extensive hepatocellular destruction and chronic tissue remodeling.

The histological improvements observed following treatment with *Cichorium intybus* are in accordance with previous reports demonstrating its hepatoprotective properties. **Nallamilli et al. (2013)** reported that administration of *C. intybus* extract promoted regeneration of hepatocytes and restoration of normal hepatic morphology in intoxicated animals. Similarly, **Riaz et al. (2024)** demonstrated that *C. intybus* treatment markedly reduced paracetamol-induced architectural disturbances, including cytoplasmic vacuolation, hepatocyte degeneration, necrosis, and inflammatory infiltration.

Khalid et al. (2018) suggested that the protective effect of *C. intybus* is associated with its ability to prevent oxidative injury and preserve hepatocyte integrity. In agreement with this hypothesis, **Li et al. (2014)** demonstrated that *Cichorium intybus* extract markedly reduced fibrosis severity, connective tissue accumulation, and architectural distortion induced by carbon tetrachloride intoxication, resulting in improved hepatic organization and absence of extensive fibrotic lesions. Additional evidence was provided by **Saggu et al. (2014)**, who showed that *chicory* fruit extract significantly reduced cirrhotic alterations, collagen deposition, hepatocyte vacuolization, portal fibrosis, and necrotic lesions induced by 4-tert-octylphenol exposure. Histological sections revealed fewer hepatic nodules, thinner fibrotic septa, and substantially improved tissue organization following treatment.

The hepatoprotective activity of *Anacyclus pyrethrum* observed in the present work is also supported by previous investigations. **Usmani et al. (2016)** demonstrated that

intoxicated animals exhibited severe architectural disruption characterized by necrosis, cellular proliferation abnormalities, eosinophilic cytoplasm, and indistinct cellular boundaries. However, treatment with *A. pyrethrum* extract produced dose-dependent improvements in liver histology. Lower doses reduced inflammatory infiltration and partially restored architecture, whereas higher doses almost completely normalized hepatic morphology, preserving normal vascular organization and hepatocyte structure. Finally, the histopathological findings indicate that paracetamol intoxication induces severe hepatic injury characterized by necrosis, fibrosis, inflammatory infiltration, steatosis, vascular alterations, and disruption of hepatic architecture. Treatment with *C. intybus*, *A. pyrethrum*, and their combination effectively attenuated these pathological alterations, suggesting potent hepatoprotective activity. These protective effects may be attributed to antioxidant, anti-inflammatory, and membrane-stabilizing properties of their phytoconstituents, which contribute to limiting oxidative damage, suppressing inflammatory responses, and promoting hepatic tissue regeneration.



Conclusion and Perspectives

Conclusion and Perspectives:

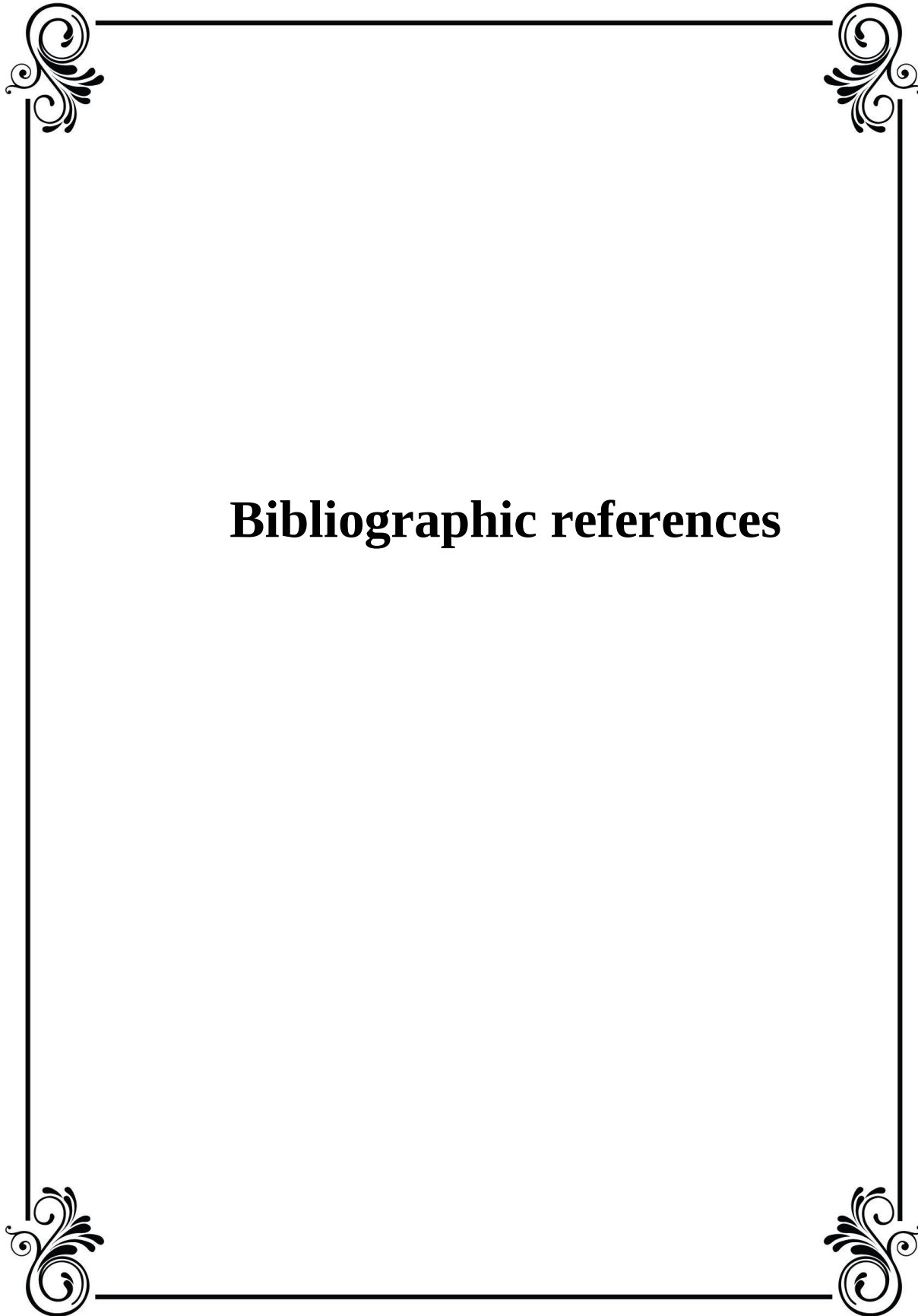
The present study demonstrated that paracetamol-induced hepatotoxicity causes significant disturbances in hepatic biochemical parameters, oxidative stress status, and liver histoarchitecture. Administration of toxic doses of paracetamol resulted in elevated serum transaminases (TGO and TGP), bilirubin, GGT, and ALP levels, accompanied by reduced total protein concentrations, confirming severe hepatic dysfunction. Moreover, paracetamol intoxication induced pronounced oxidative stress, characterized by decreased catalase activity and increased malondialdehyde levels, indicating impairment of antioxidant defenses and enhanced lipid peroxidation. Histopathological observations further confirmed hepatic injury through inflammatory infiltration, hepatocellular degeneration, and structural disorganization of liver tissue.

Preventive treatment with *Anacyclus pyrethrum* and *Cichorium intybus*, administered individually or in combination, significantly attenuated these alterations. Both plant extracts improved liver biochemical markers, restored antioxidant balance, reduced oxidative damage, and preserved hepatic architecture. Among the tested treatments, *Cichorium intybus* exhibited greater hepatoprotective activity than *Anacyclus pyrethrum*, while the combination of both extracts produced the most pronounced protective effect, suggesting possible synergistic interactions between their bioactive constituents.

The hepatoprotective effects observed in this study may be attributed to the antioxidant, membrane-stabilizing, and anti-inflammatory properties of the phytochemicals present in these plants, including phenolic compounds, flavonoids, alkaloids, tannins, and other secondary metabolites capable of reducing oxidative stress and preserving hepatocellular integrity. Despite the promising findings obtained in this study, additional investigations are necessary to further elucidate the mechanisms underlying the hepatoprotective effects of these medicinal plants. Future studies could focus on:

- Isolation and characterization of the bioactive compounds responsible for the observed hepatoprotective activity.
- Assess the subchronic and chronic toxicity profiles of both plant extracts to establish their long-term safety and tolerability

- Explore the effect of both plants on additional antioxidant enzymes and biomarkers, such as SOD, GPx, reduced glutathione (GSH), nitric oxide, and inflammatory cytokines.
- Evaluate the protective potential of these extracts against other experimental models of hepatotoxicity, including carbon tetrachloride, alcohol-induced toxicity, heavy metals, antibiotics, and other hepatotoxic drugs
- Exploration of possible synergistic interactions between *Anacyclus pyrethrum* and *Cichorium intybus* through phytochemical and pharmacological studies.
- Conduct pharmacokinetic and bioavailability studies to understand the absorption, metabolism, and tissue distribution of active constituents.
- Validation of these findings through preclinical and clinical studies to assess their therapeutic potential in human liver disorders.



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