International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Hepatoprotective Activity of *Oak* methanolic extract on paracetamol induced Albino Male Mice

Shahad Basil Ismael and Ruqaya Mohammed Al-ezzy *

Department of Molecular and Medical Biotechnology, College of Biotechnology\ Al-Nahrain University, Baghdad, Iraq.

International Journal of Science and Research Archive, 2025, 15(02), 1820–1828

Publication history: Received on 19 April 2025; revised on 28 May 2025; accepted on 31 May 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.15.2.1628

Abstract

Background: The liver, the main metabolic organ, monitors the toxicity of substances, including medications. When avoiding complications from damaging the liver, protection is crucial. Objectives: This research aimed to Detection of Quercus robur active constituent as well as determination of liver function enzyme in mic blood serum such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) after treated with paracetamol, Determination of total protein urea and creatin concentration in blood serum after treated with paracetamol and Histological evaluation of albino male mice liver after different treatment. Methods: The hepatoprotective evaluations included assessment of liver function enzyme (aspartate aminotransferase; AST and alanine aminotransferase; ALT and alkaline phosphate; ALP) in serum, as well as histopathological evaluation of liver tissue, in mice after the administration with paracetamol. Results: The results showed the extract possessed hepatoprotective ability against paracetamol liver damage by their ability to counteract paracetamol effect on AST, ALT and ALP and histological liver section. The results of AST in serum of mice were (70 ± 1.4 U\L) for negative control groups, while the level of AST enzyme (38 ± 1.1 U\L) for Oak (Quercus)treated group, for paracetamol treated group, the enzyme level was (40 ± 2.3 U\L) for drug group, and finally for interaction groups (36 ± 2.6 U\L) for Oak + paracetamol. The results of ALT were (29 ± 2.6, 23 ± 3.7, 21 ± 4.2 and 16 ± 1.1U\L) for negative control group, Quercus treated group, paracetamol treated group, and interaction group respectively. Finally, the level of ALP was (243 ± 2.8, 201 ± 2.8, 215 ± 3.5 and178 ± 0.8 U\L) for negative control, Quercus treated group, paracetamol treated group, and interaction group respectively. The level of Creatinine were (0.09 ± 0.003, 0.11 ± 0.002, 0.07 ± 0.001 and 0.09 ± 0.003 MG\DL) for negative control, Quercus treated group, paracetamol treated group, and interaction group respectively The level of Total Protein were (55 ± 0.12, 53 ± 0.14, 53 ± 0.12 and 55 ± 0.19 G\L) for negative control, Quercus treated group, paracetamol treated group, and interaction group respectively Finally, the level of Urea were (30 ± 0.01, 40 ± 0.02, 20 ± 0.001 and 30 ± 0.02 MG\DL) for negative control, Quercus treated group, paracetamol treated group, and interaction group respectively. Conclusion: Our results suggest secondary metabolite presented in plant such as (Saponins, Polyphenols, Alkaloids and Glycosides), that possessed hepatoprotective activity.

Keywords: Methanolic Extract; Mice; paracetamol; Quercus.

1. Introduction

Numerous cellular processes, such as circulation regulation, immune system support, growth signaling pathway control, and chemical metabolism, depend on the liver..(1) Although liver tissue is essential to the metabolism of many drugs and other endogenous and exogenous substances, it is frequently linked to the usage of numerous medications..(2)

When taken in standard therapeutic dosages, paracetamol, sometimes referred to as paracetamol or APAP, provides anti-inflammatory, analgesic, and antipyretic effects. For people of all ages, it is secure and easily accessible. Acute liver failure, a potentially fatal illness that may necessitate a liver transplant, might result from the urge to take larger doses

* Corresponding author: Ruqaya Mohammed Al-ezzy.

in an attempt to obtain relief more quickly..(3) Multiple phytochemicals found in medicinal plants have been utilized to treat a variety of illnesses and conditions. The phytochemical, components of these plants govern their therapeutic qualities. Natural chemical substances present in plants that can have either beneficial or detrimental impacts on health are known as phytochemicals (from the Greek word "phyton," which means plant)..(4) Numerous factors impact the process of phytochemical extraction from plant sources. Pre-extraction factors and extraction-related factors are the two groups into which these can be, separated. The portion of the plant used, its origin, particle size, moisture content, drying technique, and processing level are examples of pre-extraction factors**. The extraction method used, the solvent, is chosen, the solvent-to-sample ratio, the solvent's pH and temperature, and the extraction process's duration are all examples of extraction-related factors.(5)

The effectiveness and efficiency of phytochemical extraction are determined by these criteria taken together. Plant components were once utilized directly for therapy, but these days, sophisticated processes are used to identify, extract, and that synthesized the active ingredients in a pure form..(6) Quercus infectoria Olivier is a little tree with numerous spreading branches that, grows to a height of roughly 2.5 meters. It belongs to the Fagaceae family. (7) Quercus, also known as oak, contains a variety of bioactive compounds, including triterpenoids, steroids, phenolic acids, flavonoids, and tannins. Research on Aleppo galls (from Quercus) has confirmed several medicinal effects, including as anti-inflammatory, anti-cancer, antihypertensive, antibacterial, insecticidal, antiparasitic, monoamine oxidase-inhibitory, and anticholinesterase activities. Galls cultivated in Asia are regarded as the best due to their high tannin content. Galls are also a powerful astringent due to their high tannin content...(8) It's interesting to note that the extract from Aleppo oak galls produced the maximum output of phenolics and tannins, with around 884.79 mg of tannic acid equivalents (TAE) and 672.13 mg of gallic acid equivalents (GAE)/g, respectively. Aleppo galls prevented the growth of the harmful bacterium Yersinia enterocolitica and promoted the growth of the advantageous prebiotic Lactobacillus acidophilus bacteria. It's interesting to note that the extract from Aleppo oak galls produced the maximum output of phenolics and tannins, with around 884.79 mg of tannic acid equivalents (TAE) and 672.13 mg of gallic acid equivalents (GAE)/g, respectively. Aleppo galls prevented the growth of the harmful bacterium Yersinia enterocolitica and promoted the growth of the advantageous prebiotic Lactobacillus acidophilus bacteria.(8) High amounts of Aleppo oak galls had intriguing outcomes in experimental investigations. With roughly 884.79 mg of tannic acid equivalents (TAE) and 672.13 mg of gallic acid equivalents (GAE) per gramme of extract, the extract from Aleppo oak galls had the highest quantities of phenolics and tannins. Furthermore, the growth of the pathogenic bacteria Yersinia enterocolitica was inhibited by Aleppo oak galls, whilst the growth of the beneficial prebiotic bacterium Lactobacillus acidophilus was encouraged. Additionally, examinations of liver and kidney function, such as serum urea and creatinine levels, revealed no negative effects even after consuming large amounts of Aleppo galls extract (1000 mg/kg body weight), suggesting that it is a safe substance..).(9) Additionally, galls have been reported to have strong analgesic effects.(10) Aleppo galls have a number of therapeutic uses and could be a viable element in the treatment of paracetamol toxicity. The purpose of this study was to examine how Aleppo galls extract shielded mice against acute paracetamol toxicity. Evaluating Quercus robur's hepatoprotective effects in albino male mice treated with paracetamol is the aim of this study.

2. Materials and methods

2.1. Plant collection

Quercus robur fruits were collected from forest region Sulaymaniyah\ Iraq, during the period October_ November 2023. They were authenticated by professor Ibrahim Al jubori, Ph.D. in Medical Plants, College of Pharmacy, University of AlMustansiriya, Baghdad, Iraq. The fresh fruits of Q. robur were collected and washed throughly by tap water to remove traces and dust then let's to dry. After that they were grained to be powder.

2.2. Preparation of Plant Extract

Methaolic extract of Quercus robur was prepared by mixing (25 gm) of the plan powder with 80% methanol (250ml) at 65C for 3 hours using the Soxhlet apparatus and wait for it to cool then the plant filtered by using gouze and dry at room temperature after distribution in to Petri dish and collected in tube use

2.3. Detection of Plant Active Compound

Detection of plant active compound were detected at Biotechnology research center / Al-Nahrain university.

2.4. Preparation and doses of Drug (paracetamol)

The drug (paracetamol) used at dose (500 mg/kg) to mice by dissolving require weight in D.W

2.5. Assessment of Hepatoprotective effect

Hepatoprotective effects were assessed in albino male mice after inducing hepatic damage with paracetamol

2.6. Histopathological Evaluation of Liver

The liver was fixed in 10% formalin for 48 hours, and the procedure of (11)Camargo and Martinez and Rowe et al.(2011) was followed to prepare sections for histopathological examinations.

2.7. Statistical Analysis

The values of the investigated parameters have been represented mean ± (SE),Yet differences among means had been assessed with the aid of evaluation about difference (ANOVA) observed by means of Duncan test, the use of the pe program SPSS model thirteen.

3. Result and Discussion

- Chemical detection
- The plant contained the active constituents explained in table (1)

Table 1 Active constituent in *Quercus robur*

plant Extract	Chemical Compounds	Reagents	Indication	Results
Active constituents	Tannins	Ferric chloride	Green-blue ppt	Negative
	Glycosides	Fehlingreagent	Red-ppt	Positive
	Flavonoids	Ammonia	Yellow-ppt	Negative
		1-Shaking of extract	Foam	
	Saponines	2-Mercuric chloride	White-ppt	Positive
	Alkaloids	Myers reagent	White-ppt	positive
	Polyphenols	Ferric chloride	Green-blue ppt	positive

- Hepatoprotective potential of plant
- Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) , Alkaline phosphatase (ALP)

The results showed the extract possessed hepatoprotective ability against paracetamol liver damage by their ability to counteract paracetamol effect on AST, ALT and ALP and histological liver section. The results of AST in serum of mice was (70 ± 1.4 U\L) for negative control groups, while the level of AST enzyme (38 ± 1.1 U\L) for Oak (Quercus)treated group, for paracetamol treated group, the enzyme level was (40 ± 2.3 U\L) for drug group, and finally for interaction groups (36 ± 2.6 U\L) for Oak + paracetamol. The results of ALT were (29 ± 2.6, 23 ± 3.7, 21 ± 4.2 and 16 ± 1.1U\L) for negative control group, Quercus treated group, paracetamol treated group, and interaction group respectively. Finally, the level of ALP were (243 ± 2.8, 201 ± 2.8, 215 ± 3.5 and178 ± 0.8 U\L) for negative control, Quercus treated group, paracetamol treated group, and interaction group respectively.

Table 2 Effects of Quercus robur methanolic extract on aspartate aminotransferase; AST and alanine aminotransferase; ALT and alkaline phosphatase; ALP) in sera of albino male mice.

Group	AST (mean ±S. E) (0-40 U/L)	ALT (mean ±S. E) (0-41 U/L)	ALP (mean ±S. E) (0-270 U/L)
Negative control	70 ± 1.4 U\L	29 ± 2.6 U/L	243 ± 2.8 U/L
Plant extract	38 ± 1.1 U\L	23 ± 3.7 U/L	201 ± 2.8 U/L
Drug	40 ± 2.3 U\L	21 ± 4.2 U/L	215 ± 3.5 U/L
Plant + Drug	36 ± 2.6 U\L	16 ± 1.1 U\L	78 ± 0.8 U\L

Table (3) indicated that the level of Creatinine were (0.09 ± 0.003, 0.11 ± 0.002, 0.07 ± 0.001 and 0.09 ± 0.003 MG\DL) for negative control, *Quercus* treated group, paracetamol treated group, and interaction group respectively The level of Total Protein were (55 ± 0.12, 53 ± 0.14, 53 ± 0.12 and 55 ± 0.19 G\L) for negative control, *Quercus* treated group, paracetamol treated group, and interaction group respectively Finally, the level of Urea were (30 ± 0.01, 40 ± 0.02, 20 ± 0.001 and 30 ± 0.02 MG\DL) for negative control, *Quercus* treated group, paracetamol treated group, and interaction group respectively.

Table 3 Effects of Quercus robur methanolic extract on creatinine; total serum protein and urea in sera of albino male mice.

Group	**Creatinine (0.5-1.2 MG/DL)**	**Total protein (60-83G/L)**	**Urea (15-39 MG/DL)**
Negative control	0.09 ± 0.003	55 ± 0.12	30 ± 0.01
Plant extract	0.11 ± 0.002	53 ± 0.14	40 ± 0.02
Drug	0.07 ± 0.001	53 ± 0.12	20 ± 0.001
Plant + Drug	0.09 ± 0.003	55 ± 0.19	30 ± 0.02

Histopathological evolution of liver

The results explained in figures below:

- Mice negative control

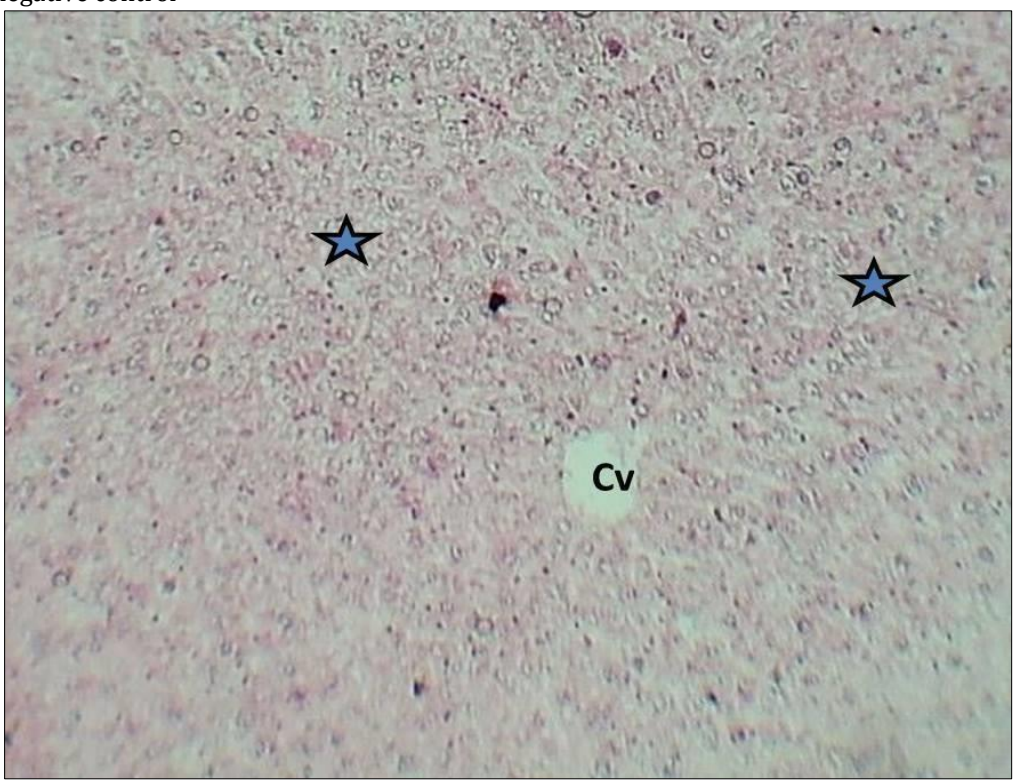

Figure 1 Section of liver (Control negative) shows: Central vein (Cv) & hepatic cords (Asterisks). H&E stain.100x

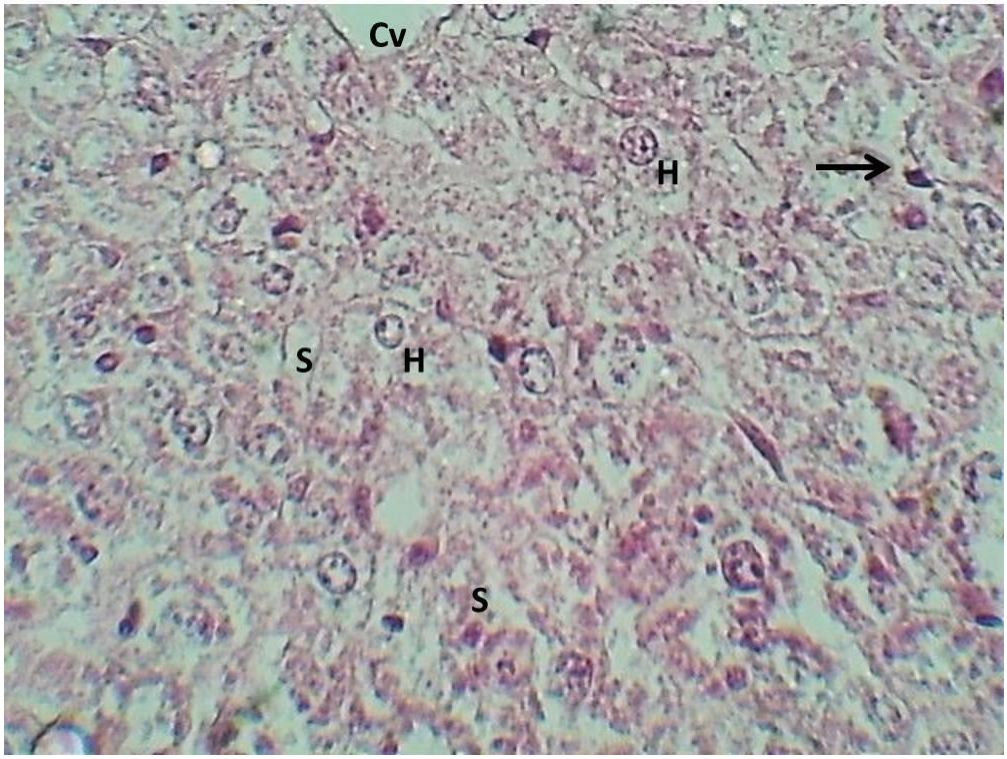

Figure 2 Section of liver lobule (Control negative) shows: Central vein (Cv), hepatocyte (H), sinusoid (S) & kupffer cells (Arrow). H&E stain.400x

- Histology of liver section after treated with oka

The histopathological figures of liver showed were showed normal appearance of hepatic cords arrangement, normal central vein, hepatocyte, and sinusoids with marked hyper cellularity of the kupffer cells (fig.3 & 4)

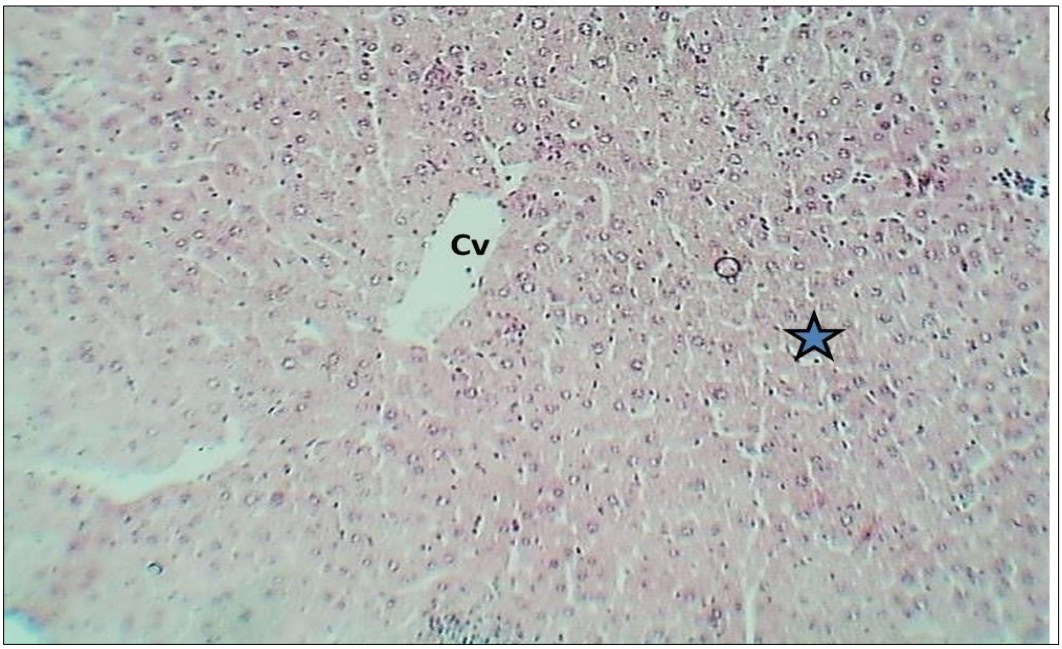

Figure 3 Section of liver (G2 plant) shows: normal appearance of the central vein (Cv) & hepatic cords (Asterisks). H&E stain.100x.

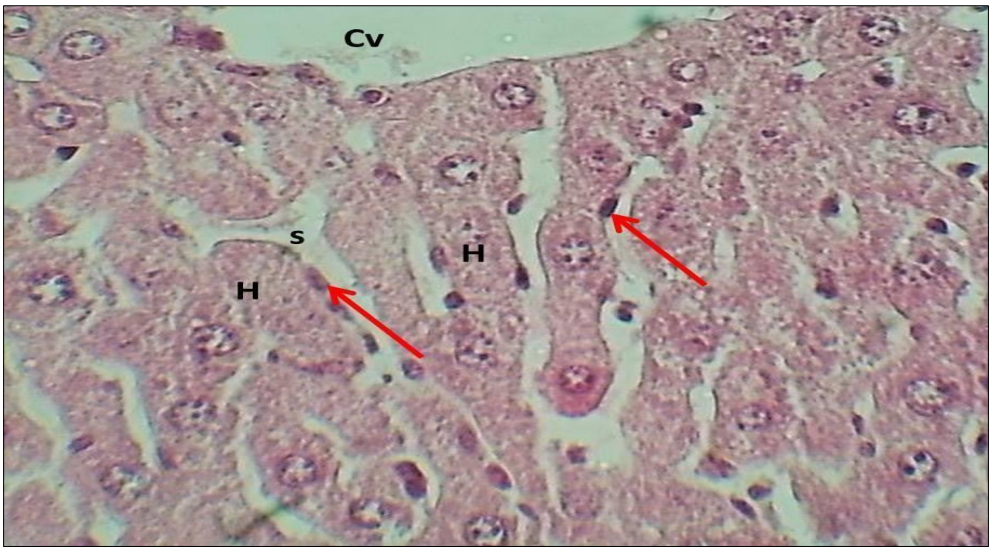

Figure 4 Section of liver lobule (G2 plant) shows: normal central vein (Cv), hepatocyte (H), sinusoid (S) & hyper cellularity of the kupffer cells (Arrow). H&E stain.400x

3.1. Liver Section of Mice After Traeted With Paracetamol

The Histopathological figures of the mice liver showed sever diffused degeneration of hepatic cords, a granular degeneration of the cytoplasmic organelles, and coagulative necrosis of hepatocytes, the portal triad showed sever vascular dilation and congestion of the portal vein, marked pre vascular cuffing associated with aggregation of lymphocytes around the blood vessels ((fig.5&6).

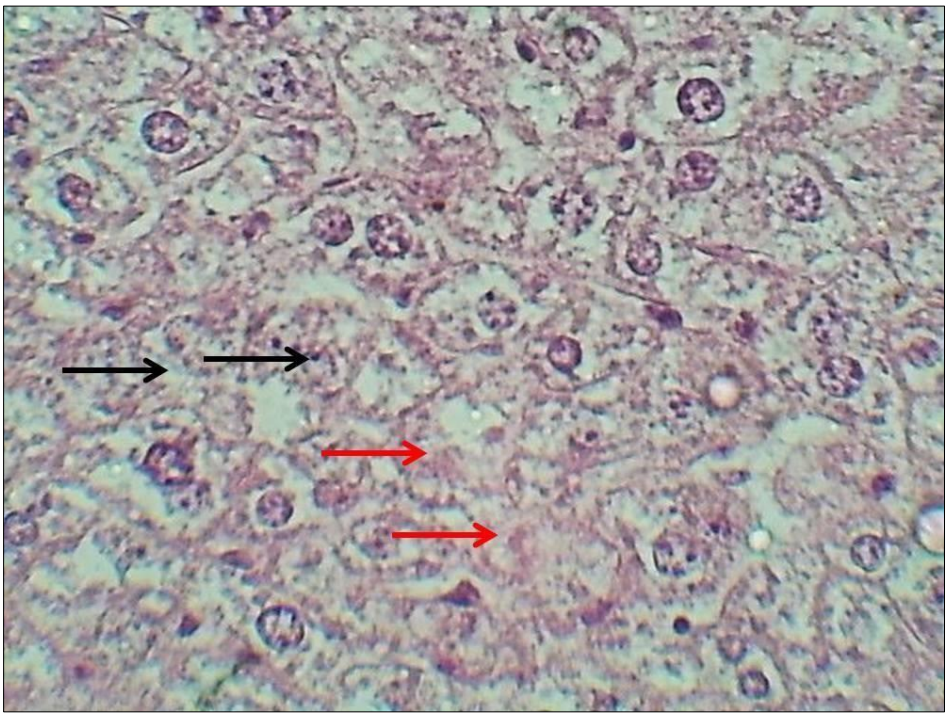

Figure 5 Section of liver (G3 Drug) shows: sever granular degeneration with the cytoplasmic organelles (Black arrows), and coagulative necrosis of hepatocytes (Red arrows). H&E stain. 400x

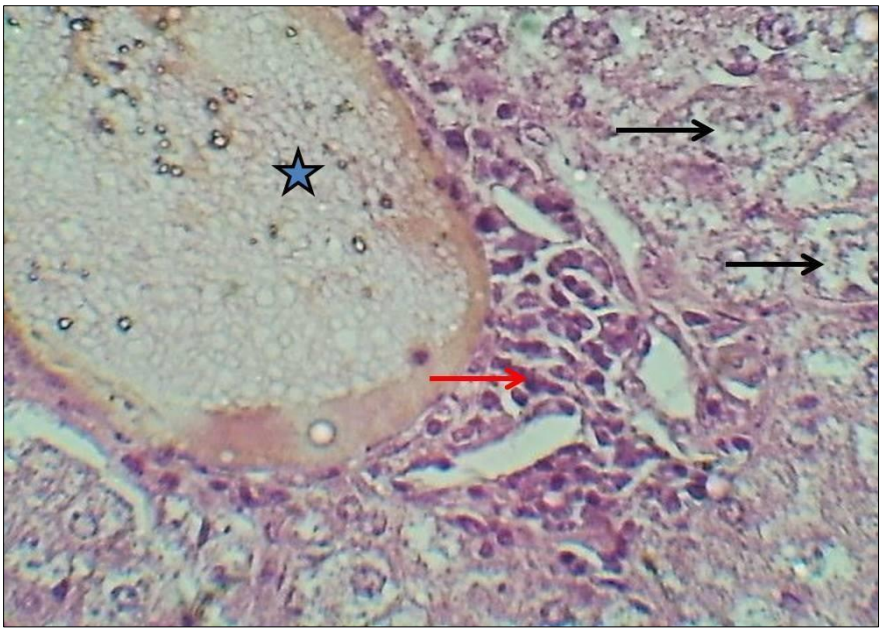

Figure 6 Section of liver (G3 Drug) shows: sever vascular dilation of portal vein (Asterisk), per vascular cuffing (Red arrow), degeneration within organelles (Black arrows) of hepatocytes. H&E stain. 400x.

- Liver Section of Mice After treated with plant

extract and Paracetamol The Histopathological figures of liver showed were showed normal appearance of hepatic cords arrangement, mild congestion of the central vein, with mild dilation of sinusoids and normal hepatocyte, and the kupffer cells (fig.7 & 8).

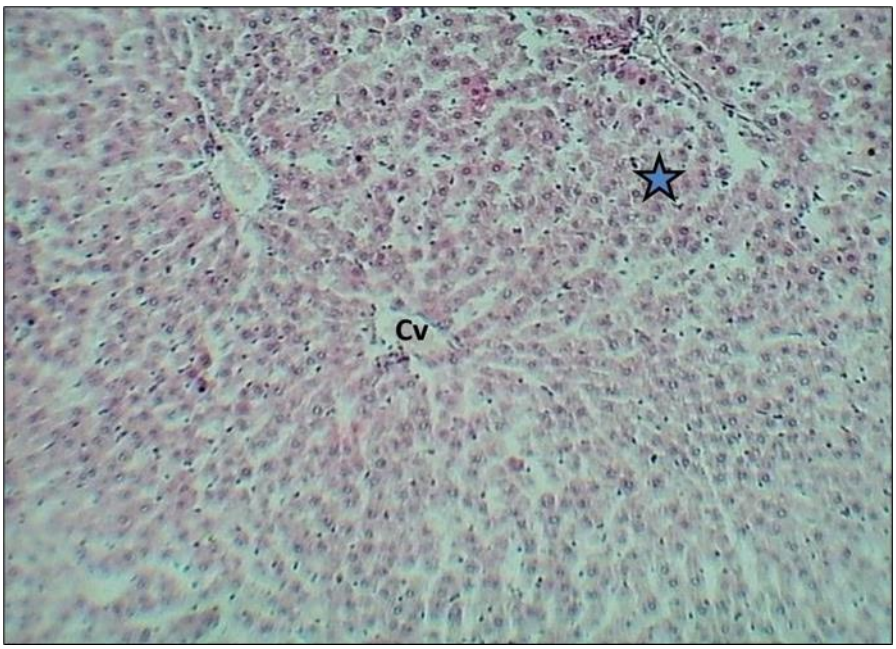

Figure 7 section of liver (G4 Plant & Drug) shows: normal appearance of the central vein with mild congestion (Cv) & normal hepatic cords (Asterisks). H&E stain.100x

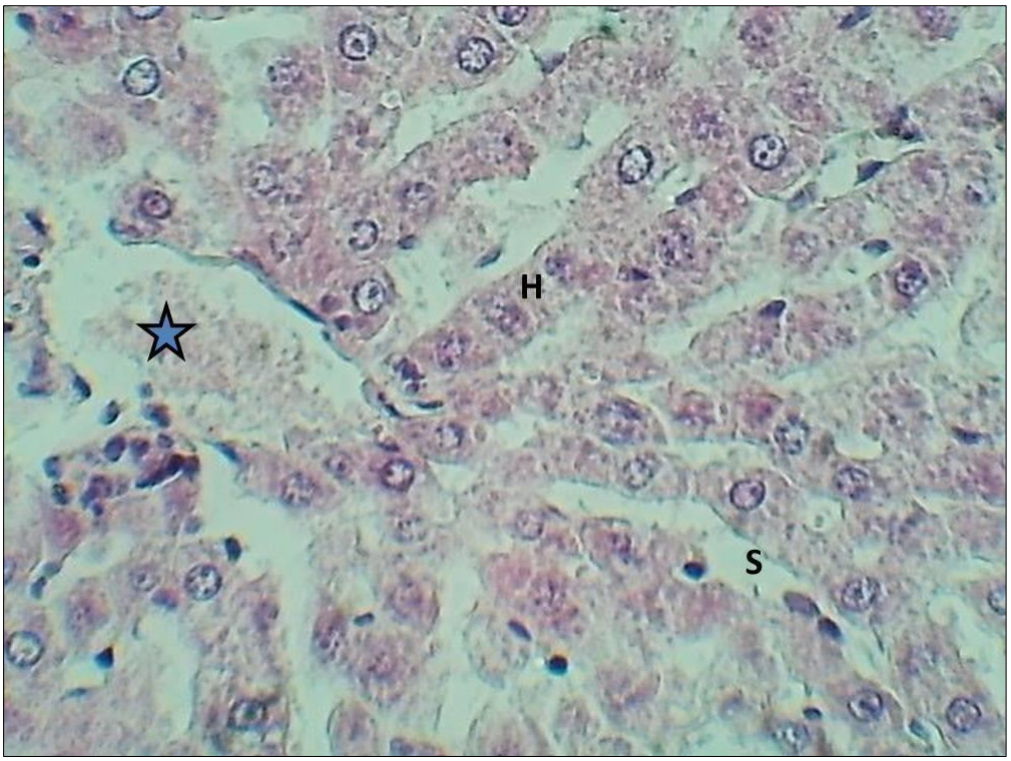

Figure 8 Section of the liver lobule (G4 Plant & Drug) shows: mild congested central vein (Asterisk), normal hepatocytes (H), sinusoid (S) & the kupffer cells. H&E stain.400x

4. Discussion

This family includes The Oak (Quercus) It is one of the most diverse groups of temperate trees, with over 500 species distributed worldwide. The members of this group possess a wide variety of combined form forms and structures. Individuals can be very long-lived (over 1000 years in some cases). In traditional medicine, many members have been used to treat and prevent various human conditions, such as gastric ulcers, diarrhea, hemorrhoids, asthma, and wound healing.(12) This research has found that the bark of oak contains a lot of bioactive compounds, including flavonoids, polyphenols, alkaloids, tannins and glycosides. They have antioxidant and antimicrobial properties which protect the plant from pathogenic organisms. Quercus species bark exhibits some of the properties of bioactive compounds that a very promising renewable materials future source. The main compounds in Quercus bark extracts include: phenolic acids (such as caffeic acid, ellagic acid, gallic acid and protocatechuic acid), tannins (including ellagitannins and roburins), and flavonoids. Q. species featured in bark extract analysis of their phenolic profiles include Q. acutissima, Q. alba, Q. macrocarpa, Q. petraea and Q. robur..(13) They have been thoroughly researched for their potential therapeutic effects on liver diseases, such as hepatitis, cirrhosis, and liver damage caused by toxins or medications.

These results are consistent with recent research showing that natural flavonoids, such as kaempferol and naringenin, or flavonoid glycosides, found and measured in Quercus species galls, demonstrated hepatoprotective effects in a number of animal species because of their anti-inflammatory, anti-apoptotic, and antioxidant properties.(14)

Acetaminophen, often known as paracetamol, is a common over-the-counter drug used to treat fever and pain. (15). While it is generally considered safe when used as directed, excessive or prolonged use of paracetamol

ol can lead to liver damage(16). the risk of paracetamol-induced liver damage is influenced by various factors, including the dose of paracetamol ingested, individual susceptibility, and the presence of other risk factors such as chronic alcohol consumption or pre-existing liver disease(17).

5. Conclusion

All these results obtained attributed to plant secondary metabolite presented in plant such as (Saponins, Polyphenols, Alkaloids and Glycosides), that possessed hepatoprotactive activity.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Zmora N, Bashiardes S, Levy M, Elinav EJCm. The role of the immune system in metabolic health and disease. 2017;25(3):506-21.

[2] Zimmerman HJ. Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver. 1999.

[3] Dogaru G, Bulboaca AE, Gheban D, Boarescu PM, Rus V, Festila D, et al. Effect of liposomal curcumin on acetaminophen hepatotoxicity by down-regulation of oxidative stress and matrix metalloproteinases. 2020;34(2):569-82.

[4] Bajpayee KK, Ashaq MJJOS, RESEARCH, TEACHING. MEDICINAL PLANTS USED IN DIFFERENT DISEASES. 2024;3(5):55-68.

[5] Benchiha W, Mahroug S, Bouterfas K, Moulessehoul YJEAJoBSC, Physiology, Biology M. In Vitro Antifungal Activity of The Flavonoid extracts from Rhamnus alaternus L. (Rhamnaceae). 2022;14(2):43-52.

[6] La Mesa C, Corbo A, Gkouvi A, Risuleo GJAROIC. Bio-active principles from the animal and plant kingdom: A review. 2020; 1:1-12.

[7] Shakir MA, Mahmood INJIJoFM, Toxicology. Hyperlipidemia that Induced in Male Rats and Role of Flavonoids Extract of Quercus infectoria in Treatment. 2020;14(1).

[8] Abdallah AAM, Albadawi EA, Aboonq MS, Desouky MK, Ahmed AR, Bafail R, et al. Aleppo galls alleviate paracetamol-induced hepatotoxicity and tissue damage: An experimental study. 2023;14(1):1.

[9] Banc R, Rusu ME, Filip L, Popa D-SJP. Phytochemical profiling and biological activities of quercus sp. galls (Oak galls): a systematic review of studies published in the last 5 years. 2023;12(22):3873.

[10] Soltanifard R, Nahidi F, Mojab F, Birjandi MJJoHP. The effect of Quercus infectoria pair cream on the severity of episiotomy pain in nulliparous women. 2021;10(4):401-7.

[11] Rodriguez-Diaz J, Rosas-Camargo V, Vega-Vega O, Morales-Espinosa D, Mendez-Reguera A, Martinez-Tlahuel J, et al. Clinical and pathological factors associated with the development of hepatocellular carcinoma in patients with hepatitis virus-related cirrhosis: a long-term follow-up study. 2007;19(3):197-203.

[12] Krutovsky KV, Popova AA, Yakovlev IA, Yanbaev YA, Matveev SM. Response of Pedunculate Oak (Quercus robur L.) to Adverse Environmental and Weather Conditions in Genetic and Dendrochronological Studies. 2024.

[13] Elansary HO, Szopa A, Kubica P, Ekiert H, Mattar MA, Al-Yafrasi MA, et al. Polyphenol profile and pharmaceutical potential of Quercus spp. bark extracts. 2019;8(11).

[14] Lu R, Yu R-J, Yang C, Wang Q, Xuan Y, Wang Z, et al. Evaluation of the hepatoprotective effect of naringenin loaded nanoparticles against acetaminophen overdose toxicity. 2022;29(1):3256-69.

[15] Ayoub SSJT. Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. 2021;8(4):351-71.

[16] Wróblewski T, Kobryń K, Kozieł S, Ołdakowska-Jedynak U, Pinkas J, Danielewicz R, et al. Acetaminophen (Paracetamol) induced acute liver failure–A social problem in an era of increasing tendency to self-treatment. 2015;22(4):762-7.

[17] Teschke R, Zhu YJSPT. Paracetamol (acetaminophen), alcohol, and liver injury: Biomarkers, clinical issues, and experimental aspects. 2018; 1:113.