Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Biochemical and Histological Study of Rat Liver Injury Induced by carbon tetrachloride (CCl_4)

Ansam Awad Ali[1]*, Mohammed Abbas jasim[2]

[1,2]Department of Biology, Education College for Women, University of Anbar, IRAQ

*Corresponding Author: Ansam Awad Ali

DOI: https://doi.org/10.31185/wjps.610
Received 20 November 2024; Accepted 24 December 2024; Available online 30 March 2025

ABSTRACT: The liver is a vital organ that plays a key role in the detoxification of endogenous and exogenous, current study aimed to evaluate the toxic effect of carbon tetrachloride (CCl4) on the liver structure substances, thirty-four male Sprague Dawley rats at the age of (8-10 weeks) were used. The experiment was carried out by dividing the animals into six groups (T1, T2, T3, T4, T5A, T5B, T5C, T6), and each group contained five animals. Liver damage was stimulated in the groups (T1, T2, T3) synergistically between the damage-inducing substance CCl4. The amino acid BABA is in different concentrations (100, 150, 200) for BABA for five weeks in two doses. In the fourth group, T4, or giving it only CCl4 also twice a week for five weeks. As for the fifth group, T5, which was divided into (A, B, and C), it was given only the amino acid BABA in different concentrations (100, 150, 200). Nine animals were placed in this group, three animals for each group, while keeping the sixth group, T6, for control, as it was given only a physiological solution. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin and histology of the liver were evaluated. The ALT, AST and bilirubin showed high mean levels in T4 group (132.8±28.09,138.9±6.09, 3.88±1.60), respectively. while showed low level in T5C group (93.7±5.03,68.9±5.52, 2.12±0.93) and control group (91.2±4.87 ,70.1±3.15, 0.91±0.46), respectively. A section of the liver of group (T4) rats treated with carbon tetrachloride, showed the pathological anatomical shapes of the hepatic lobules showed severe congestion in the portal vein, and a noticeable disorder in the hepatic cord characterized by severe fatty degeneration of the liver cells with Atrophy, congestion and congestion of central veins, portal vessels and normal liver cells. The current study concluded the relationship between the increase in liver enzyme in the case of treatment with CCl4 and its decrease in treatment with the amino acid BABA. High dosages of CCl4 exhibited liver function impairment due to heightened levels of enzymatic biomarkers, specifically ALT and AST, which correlate with the extent of liver damage and dysfunction.

Keywords: Rat liver, BABA. ALT, AST

1. INTRODUCTION

Carbon tetrachloride (CCl4) is a well-known hepatotoxic agent that induces rapid liver damage, advancing from steatosis to centrilobular necrosis. The prolonged dose of CCl4 induces chronic liver damage and is a well-established paradigm for inducing hepatic fibrosis (1). CCl4 is recognized for its ability to generate reactive oxygen species, deplete glutathione in phase II enzymes, and diminish antioxidant enzymes and substrates, inducing oxidative stress, a significant contributor to both acute and chronic liver injury (2).

The liver injury caused by CCl4 results from free radicals and lipid peroxidation, leading to hepatic cell destruction. CCl4 necessitates bioactivation via the phase I cytochrome P450 system in the liver to generate the reactive trichloromethyl radical (CCl•3) and the proxy trichloromethyl radical (•OOCCl3). Free radicals can interact with polyunsaturated fatty acids (PUFAs) to form alkoxy (R•) and peroxy radicals (ROO•), which then lead to the production

*Corresponding author: ans22w4004@uoanbar.edu.iq

of lipid peroxides, inflict damage on cell membranes, alter enzyme performance, and ultimately result in hepatic injury or necrosis (3).

The liver is a vital organ that plays a crucial role in metabolic balance. It possesses remarkable regenerating capacity following hepatic mass reduction (4). Iredale et al. (5) classified liver fibrosis as a pathological state marked by excessive collagen deposition and other extracellular matrix components. The authors noted that liver fibrosis was first deemed progressive and irreversible; however, numerous clinical investigations have demonstrated that it can be reversed if the underlying causes are effectively eliminated or if patients receive appropriate treatment.

β-amino butyric acid (BABA) is one of the rare free compounds found in nature, It is a non-protein amino acid that has been shown to have protective effects and enhance the body's cell defenses against infection (6, 7). The impact of BABA on animal cells has been recently discussed. In one study including healthy male Sprague Dawley mice, it was observed to alter blood parameters and immune function. Research indicates that BABA facilitates wound healing and diminishes inflammation. Furthermore, BABA induces B cells to produce IgG and IgM antibodies, which then activate the complement system and enhance the interaction between innate and adaptive immunity (8). In contrast, the adaptive immune response functions over an extended timeframe and is influenced by prior encounters. It exhibits high specificity at the molecular level, frequently directed towards a specific microbial species, thanks to antigen-specific receptors formed through somatic DNA recombination within lymphoid clones. Therefore, the current study aimed to evaluate the toxic effect of carbon tetrachloride (CCl4) on the structural materials of the liver, and to study the protective effect of BABA on damaged tissues.

2. MATERIALS AND METHODS

2.1 Chemicals

Beta aminobutyric acid (BABA) (Germany) and CCl4 (UK) were used in the study.

2.2 Laboratory analyses

2.2.1 Blood sample collection

This experiment was carried out using 34 male Sprague Dawley rats weighing between (130-150) g and aged between (8) weeks were purchased from the animal house at the Iraqi Center for Medical Cancer Research at Al-Mustansiriya University - Baghdad - at the age of (8-10 weeks). All rats were in good health, with weights ranging from 140 to 160 grams, and they were placed in The animal laboratory of the College of Education for Girls, Anbar University, and laboratory animals were placed inside plastic cages with covers. Special metal mesh for raising rats. The laboratory conditions were suitable for living, and the temperature was set at 25 °C, and the duration of ventilation and lighting was adjusted day/night. The animals remained for 12 days before the start of the experiment, which was conducted during the month of June, and the animals were fed using a special diet. After ensuring that the animals were anesthetized, the blood samples were obtained from the abdominal aorta and stored in sterile tubes containing an anticoagulant (K3-EDTA), In order to examine the hematological parameters

2.3 Distribution of animal's groups

The experiment was carried out by dividing the animals into six groups (T1, T2, T3, T4, T5A, T5B, T5C, T6), and each group contained five animals. Liver damage was stimulated in the groups (T1, T2, T3) in a synergistic manner between the damage-inducing substance CCl_4. And the amino acid BABA in different concentrations (100, 150, 200) for BABA for five weeks in two doses. In the fourth group, T4, or giving it only CCl_4 also twice a week for five weeks. As for the fifth group, T5, which was divided into (A, B, and C), it was given only the amino acid BABA in different concentrations (100, 150, 200). Nine animals were placed in this group, three animals for each group, while keeping the sixth group, T6, for control, as it was given only a physiological solution. It was left as a control for liver damage. The groups were divided as follows.

First group, T1, was injected with CCl_4 to stimulate liver damage at a concentration of 0.8 and BABA at a concentration of 100 mg/kg. The weights of the animals in this group were all 140 grams per animal.

Second group, T2, was injected with CCl4 at a concentration of 0.8 and BABA at a concentration of 150 mg/kg, noting that the weights of the animals in this group were 140 grams for each animal.

Third group, T3, was injected with CCl4 to stimulate liver damage at a concentration of 0.8 and BABA at a concentration of 150 mg/kg, as the weights of this group were 140 grams for each animal.

Fourth group, T4. This group was injected only with CCl4 to stimulate liver damage, at a concentration of 0.8 mg/kg, as the weights of this group were 150 grams per animal.

Fifth group, T5, is the negative control group, which was divided into three groups with different concentrations of the amino acid BABA, as follows:

Fifth group, T5A. This group was injected with BABA at a concentration of 100 mg/kg, and the weight of this group was 140 grams for each animal.

Fifth group, T5B. This group was injected with BABA at a concentration of 150 mg/kg, as the weights of this group were 145 grams for each animal.

Fifth group, T5C, was injected with BABA at a concentration of 200 mg/kg, as the weights of this group were 150 grams for each animal.

Sixth group, T6. This group was left to the positive control, where NORMAL SALINE was injected with two injections per week, 1mL for each animal, and the weights of this group were 160 for each animal.

2.4 Biochemical analysis

2.4.1 Measurement of serum ALT, AST and bilirubin

Blood samples were centrifuged at 3000 rpm for 5 min. Sera were collected, and the levels of ALT, AST and bilirubin were measured using a Cobas Mira Plus CC Chemistry Analyzer (Switzerland).

2.5 Histopathological examination

After drawing blood samples, the animals were dissected directly by making an incision in the abdominal cavity from the bottom upwards towards the heart, then the liver were removed after removing the fatty tissue and the surrounding connective tissue, then washed with distilled water to remove the blood on them, then dried by placing them on filter paper and weighing them. These tissue are preserved in a 10% formalin solution. Following mounting, a 5µm slice of each tissue was cut and subsequently immunostained with H&E. Two pathologists performed the histopathological grading using a semiquantitative scale: normal = 0, mild = <25%, moderate = 25–50% and severe = >50% of affected area (9).

2.3 Statistical Analysis

Data were presented as the mean ± standard deviation (SD). Group differences were assessed using one-way analysis of variance (ANOVA). Post hoc testing for intergroup comparisons was conducted utilizing the Least Significant Difference (LSD) test, with a P value <0.05 being significant.

3 RESULTS AND DISCUSSION

The results showed a significant difference ($P \leq 0.05$) in the studied liver parameters of injured rats. The ALT(alanine aminotransferase), AST (aspartate aminotransferase) and bilirubin showed high mean levels in T4 group (132.8±28.09,138.9±6.09, 3.88±1.60), respectively. while showed low level in T5C group (93.7±5.03,68.9±5.52, 2.12±0.93) and control group (91.2±4.87 ,70.1±3.15, 0.91±0.46), respectively as showed in table (1), Figures (1,2, and 3).

Table (1): Mean levels of liver enzyme in studied rats groups

Groups	ALT	AST	Bilirubin
T1	122.2 ±3.11a	108.0±3.39 b	3.10 ±0.90a
T2	112.3±4.57 b	102.0±2.52 b	2.58 ±0.93a
T3	97.0±9.92 b	93.5±5.08 c	3.54±0.98a
T4	132.8±28.09 a	138.9±6.09 a	3.88±1.60a
T5A	97.7±3.21 b	78.0±2.02 d	3.07±1.48a
T5B	99.3±2.52 b	75.0±7.09 d	2.24±1.51a
T5C	93.7±5.03 c	68.9±5.52 e	2.12±0.93a
T6(control)	91.2±4.87 c	70.1±3.15 e	0.91±0.46b
P value	**0.001****	**0.001****	**0.01****

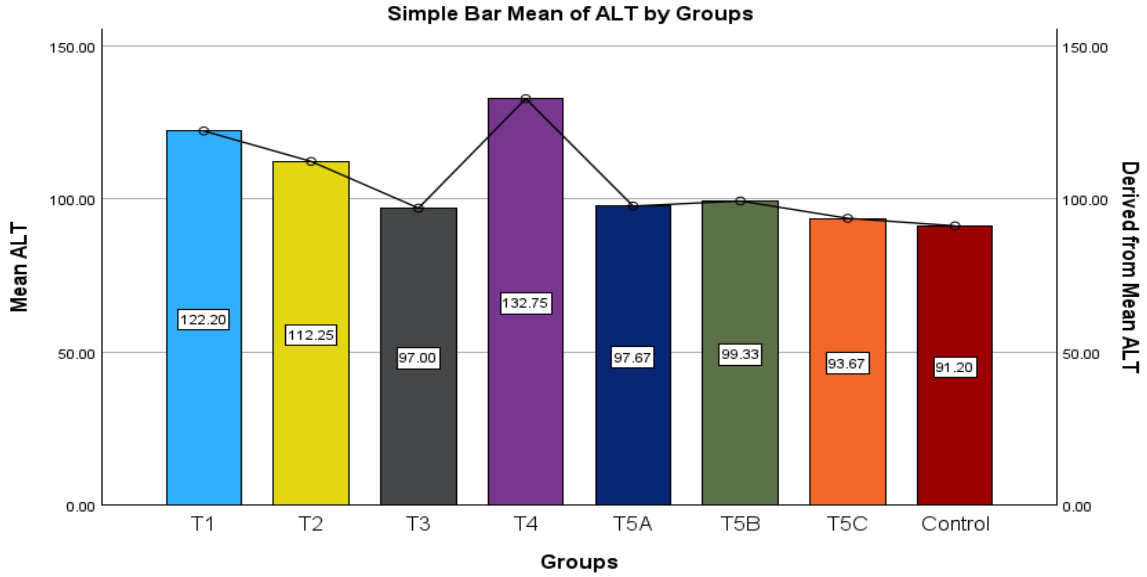

Figure (1): Mean levels of ALT in studied rats groups

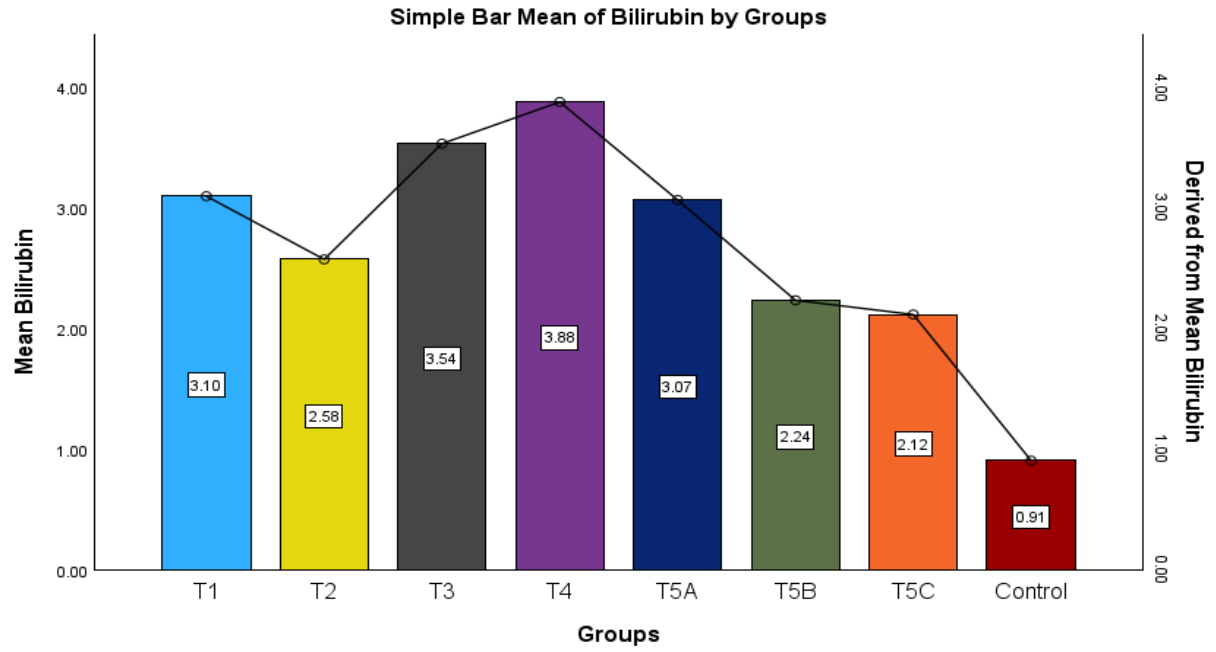

Figure (2): Mean levels of AST in studied rats groups

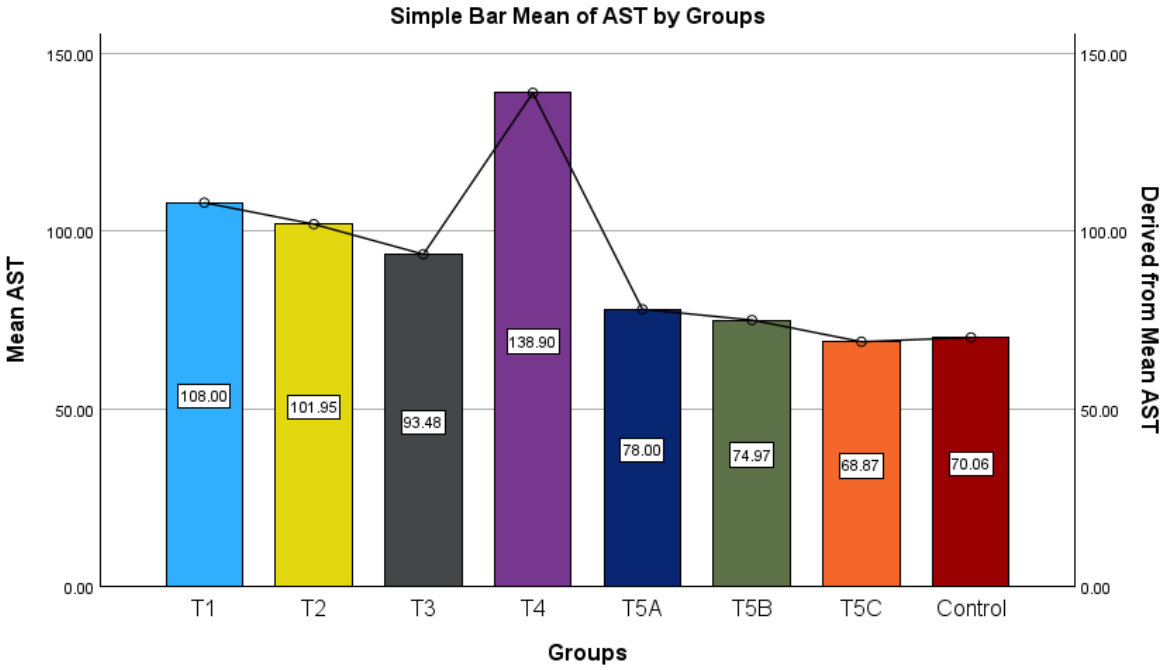

Figure (3): Mean levels of Bilirubin in studied rats groups

Correlation between liver parameters in studied rats

Table (2) showed a positive correlation between bilirubin and ALT(0.408*), bilirubin and AST(0.459**) and between ALT and AST(0.769**).

Table (2): Correlation between liver parameters in studied rats

		Bilirubin	ALT	AST
Bilirubin	**Pearson Correlation**			
	Sig. (2-tailed)			
	Sig. (2-tailed)	**.001**		
ALT	**Pearson Correlation**	**.408***	**--**	
	Sig. (2-tailed)	**.020**		
AST	**Pearson Correlation**	**.459****	**.769****	**--**
	Sig. (2-tailed)	**.008**	**<.001**	

Histological study of the injured rats liver induced with BABA and CCL4

Microscopic examination of histological sections of the livers of control group animals showed that the liver is surrounded by a liver capsule and the liver parenchyma is divided into lobules. The lobule is a hexagonal prism with a branch of the hepatic vein located in the middle (CV) Central vein, and hepatocytes (H) form sheets that radiate towards the periphery. In a hexagonal corner (but not in every corner) there are portal areas. The presence of sinusoids (S) and Coover cells is also observed normally as showed in Figure (1).

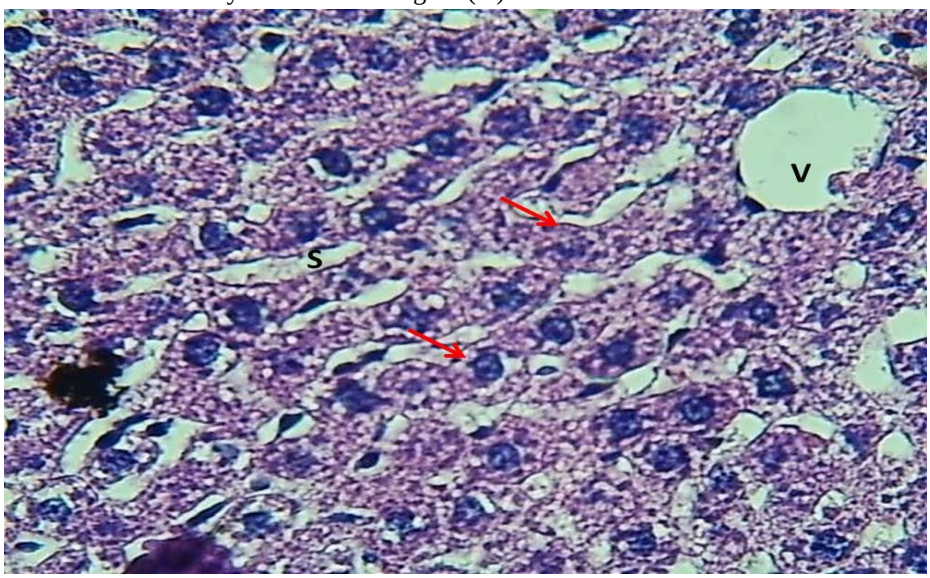

Figure 4: Section of hepatic lobule (control group) showing normal central vein (V), normal portal area (P) and normal arrangement of hepatic cords (asterisk).H&E.400x.

Figure 2, also show a section of the liver of group (T1) rats treated with BABA + carbon tetrachloride, where the pathological histopathological shapes of the hepatic lobules revealed a noticeable widening with congestion of the portal vein, and the formation of many Choledochal cysts, severe hepatocyte steatosis and hypertrophic adipogenesis.

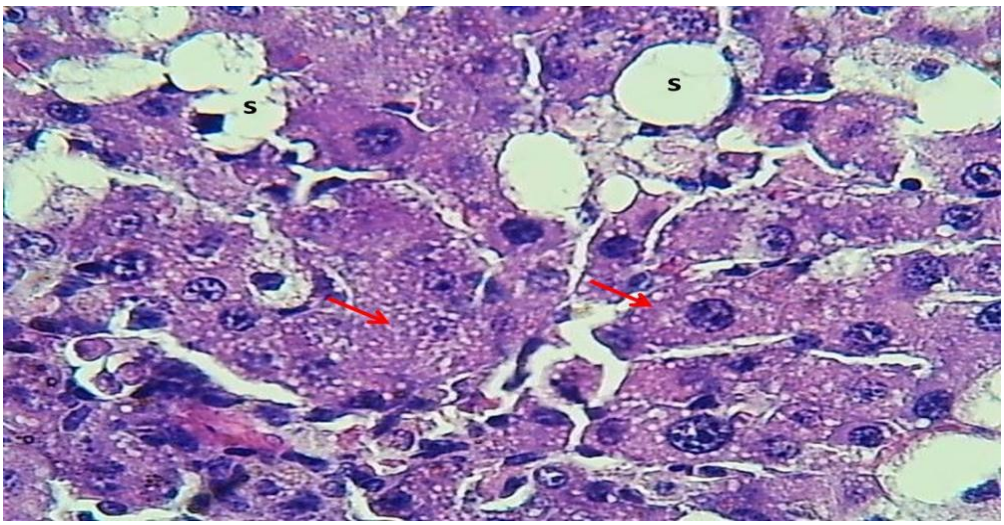

Figure 5: Section of hepatic lobule (T1-2) showing severe hepatocyte steatosis (arrows) with adipocyte hyperplasia (S) H&E.400x

Figure 3, showed, a section of the liver of group (T2) rats treated with beta-amino butyric acid (BABA) + carbon tetrachloride (CCl4) showed the histopathological shapes of the liver lobules with noticeable widening with central vein congestion, and the parenchyma of the lobules showed fatty degeneration.

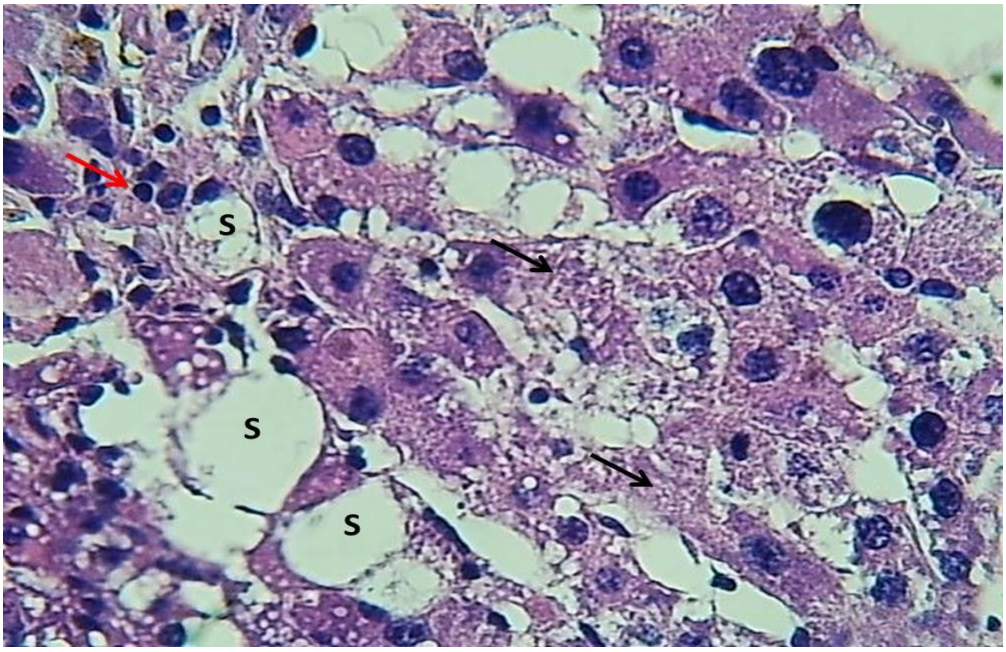

Figure 6: Section of hepatic lobule (T 2) showing hepatitis with marked steatosis (S) with hepatocyte necrosis (black arrows) with leukocyte infiltration (red arrow). H&E.400x

Figure 4, showed, a section of the liver of group (T3) rats treated with BABA + CCl4. Histopathological figures of the hepatic lobule revealed moderate hepatitis characterized by mild central vein dilatation, marked degeneration in the pericentral region and areas with mild necrosis of hepatocytes which showed normal central veins and portal triad and normal arrangement of hepatic cords which showed few degenerative and necrotic changes with little infiltration for white blood cells.

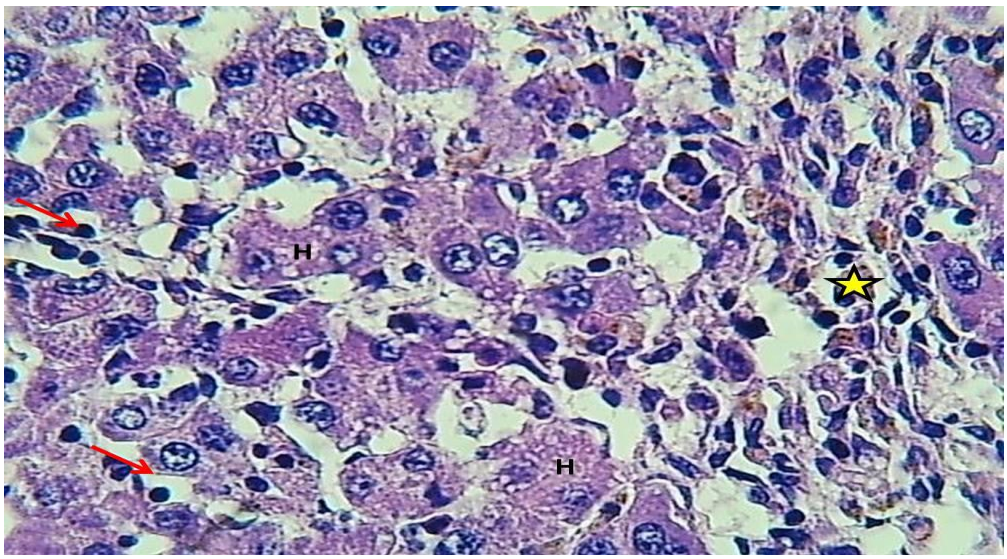

Figure 7: Section of a hepatic lobule (T 3) showing mild hepatitis with marked fibrosis (asterisk) with mononuclear leukocyte infiltration (arrows), and hepatocyte degeneration and necrosis (H) H&E 400x.

Figure 5, showed a section of the liver of group (T4) rats treated with CCl4, the pathological anatomical shapes of the hepatic lobules showed severe congestion in the portal vein, and a noticeable disorder in the hepatic cord characterized by severe fatty degeneration of the liver cells with Atrophy, congestion and congestion of central veins, portal vessels and normal liver cells.

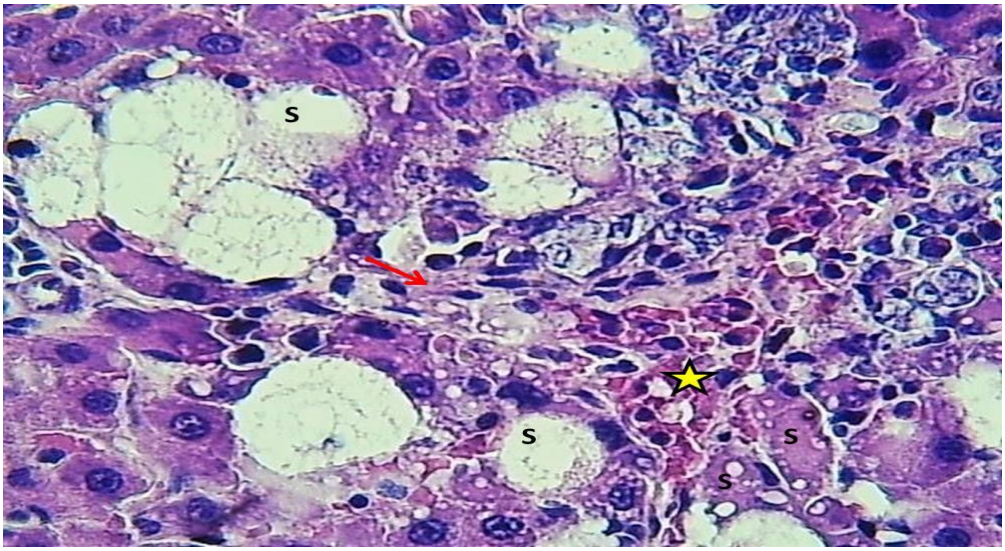

Figure 8: Section of a hepatic lobule (T4) showing severe hepatocyte steatosis (S) with minimal fibrosis (arrows), and congestion with mononuclear leukocyte infiltration (asterisk).H&E.400x

A section of the liver of group (T5A) rats treated with BABA, the pathological anatomical shapes of the hepatic lobules in this group showed the normal arrangement of the hepatic cords with normal liver cells. Changes in these groups were limited to mild congestion of the central vein and sinusoids and mild hypercellularity of Kupffer cells as showed in Figure 6.

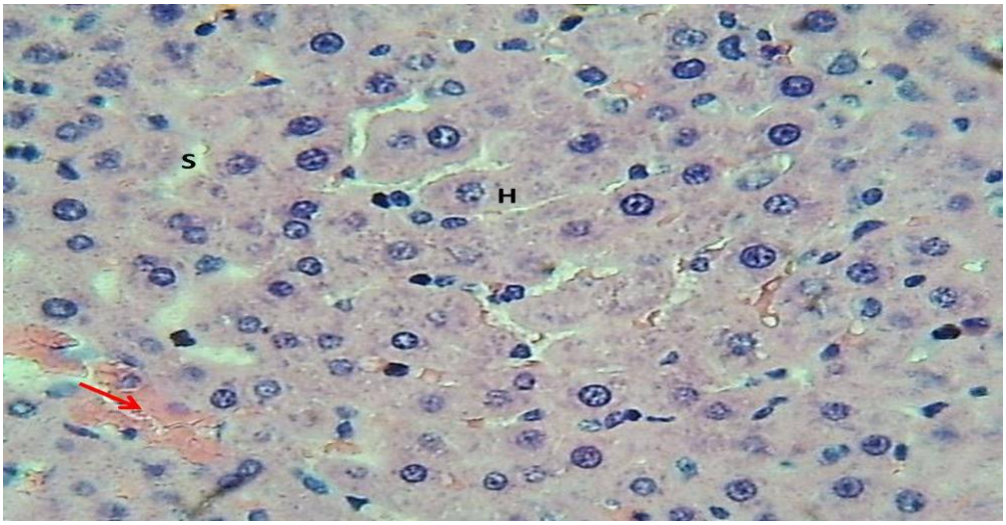

Figure 9: Section of hepatic lobule (T5A1) showing mild sinusoidal congestion (arrow) with normal hepatocytes (H) H&E.400x

Figure 7, showed, a section of the liver of group (T5B) rats treated with BABA. The histopathological shapes of the liver lobules show a normal central vein with moderate peripheral and zonal cellular swelling of the liver cells.

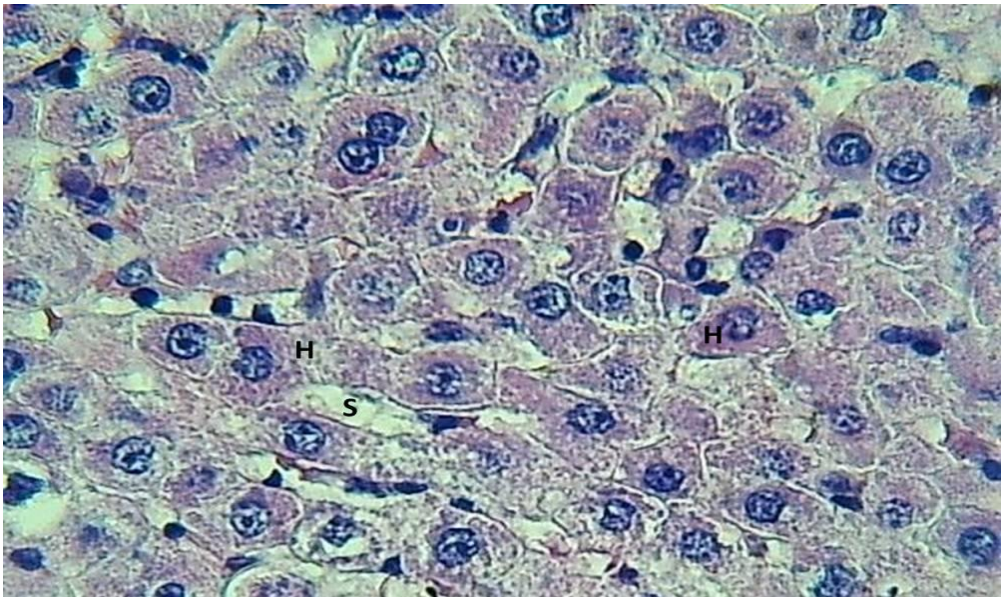

Figure 10: Section of the hepatic lobule (T5B2) showing the normal state of the hepatocytes (H) and sinusoids (H&E.400x (S).

A section of the liver of group (T5C) rats treated with BABA, the pathological anatomical shapes of the liver lobules showed mild congestion in the central vein and the portal vein, with a normal arrangement of liver cells and similar to histological sections. In this case, the tissue sections are largely from the control group, and this indicates that the BABA compound has many protective effects, Figure 8.

Despite the increase in BABA concentration, the histological structure examination showed normal, and this indicates that the doses of beta-aminobutyric acid (BABA) did not affect the histological structure of the livers of the male rats used in the experiment.

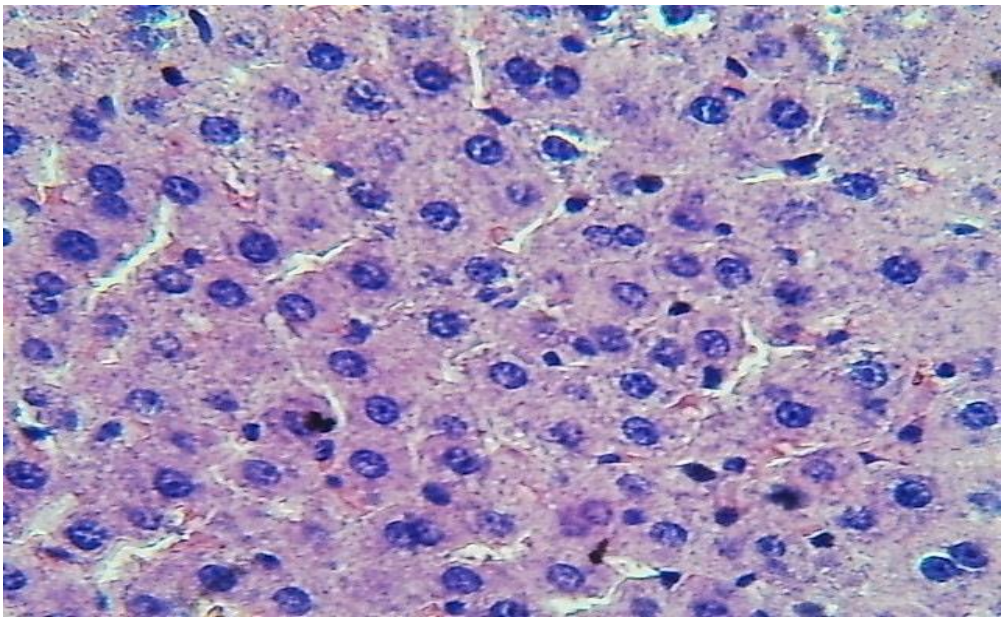

Figure 11: Section of the hepatic lobule (T5C1) showing the normal state of hepatocytes (H) and sinusoids (S) H&E.400x

Amino acids such as BABA can help fight many diseases and have the ability to reduce liver tissue damage (10). The reason may be attributed to the protective effect of the beta-amino butyric acid BABA in removing toxins from the liver and it can treat liver injury disorders, as it works to reduce fat peroxidation and restore the state of antioxidants to a normal state (11), or the reason may be due to its synthesis and renewal of the glutathione compound, which plays a role

in The latter plays an important role in protection against toxins caused by reactive oxygen species (12). Research has shown that beta-aminobutyric acid plays a beneficial function in accelerating wound healing and reducing inflammation. In addition, BABA stimulates B cells to generate Igg and IgM antibodies, which in turn activate the complement system and facilitate communication between innate and acquired immunity (13). The BABA compound also plays its role in protecting cells from toxicity. Carbon tetrachloride induced by trapping free radicals or increasing levels of antioxidant enzymes (14).

The results of this study are also consistent with a study conducted by Al-Dulaimy & Jasim, (1), which used different concentrations (100, 200, 300) mg/kg of the non-protein amino acid BABA in studying its effect on the livers of mice. The results indicated the possibility of using BABA as an antioxidant. Vital, as the amino acid facilitated the normal preservation of Toll-like receptor 4 levels while also stimulating and activating it, even at elevated concentrations, so positively influencing the enhancement of immunity in the animals utilized in the investigation.

Also, recent studies have demonstrated that the amino acid has a role in increasing the number of white blood cells and lymphocytes and increasing the production of IgG in rats treated with the amino acid BABA (15).

EL Sayed *et al*. (4) noted that CCl4 is recognized for its hepatotoxic effects. It was observed that it induces acute liver injury, including necrosis and steatosis. This effect results from the generation of free radicals, which can produce lipid peroxides that may lead to cellular membrane damage, alterations in enzymatic activity, and ultimately induce liver injury and necrosis. Höhme *et al*. (16) indicated that CCl4 initially caused cell death in a pericentral ring of hepatocytes, subsequently leading to the degradation of the distinctive microarchitecture of the hepatic lobules. UskokovićMarković *et al*. (17) shown that CCl4-induced liver necrosis serves as a model for experimental hepatic necrosis and cirrhosis resulting from oxygen free radicals. The study conducted by Lindqvist *et al*., (18) showed that BABA intake played a protective role against cadmium-induced lipid peroxidation and liver and kidney injury in mice. In another study conducted by (19) it was reported that antioxidants such as BABA were effective against liver toxicity or nephrotoxicity caused by CCl4 and reducing oxidative stress. BABA, also known as a cell membrane antioxidant, reduces oxygen molecules causing oxidative stress (20).

A study by Liu et al. (21) demonstrated that CCl4 is believed to initiate free radical-mediated lipid peroxidation, resulting in the accumulation of lipid-derived oxidation products that induce liver injury and excessive collagen deposition, ultimately leading to liver fibrosis. Several researchers have previously shown that antioxidants mitigate CCl4 toxicity, especially hepatotoxicity, by preventing lipid peroxidation, reducing ALT and AST activities, and enhancing antioxidant enzyme activity.

The study showed high toxic effect of CCl4 on liver, this is due to its inhibition of the metabolic enzyme system in the liver, which contains sulfhydryl groups, and the disengagement of oxidative phosphorylation in mitochondria (22). Carbon tetrachloride also contributes to increased lipid peroxidation, DNA damage, and depletion of sulfhydryl groups. , altered calcium balance, and liver congestion (23). This may be due to the high level of GGT, LDH, and ALP enzymes in the serum due to the effect of beta-aminobutaric acid in damaging the cell membrane and the leakage of these enzymes from the cytosol in the liver into the bloodstream, which gives an indication of the effect of this acid in hepatotoxic events (24). The reason for the increase in fats such as cholesterol, LDL, and VLDL may also be due to acid, which leads to an imbalance of antioxidants in the liver (25).

4 CONCLUSION

The current study concluded the relationship between the increase in liver enzyme in the case of treatment with CCl4 and its decrease in treatment with the amino acid BABA. At high doses of CCl4, there was an impairment of liver function as a result of heightened levels of enzymatic biomarkers such as ALT and AST, which are related with the degree of liver damage. Additionally, there was an impairment of liver function as a consequence of elevated biomarker levels.

REFERENCES

[1] Mousa MA, Elhagrasi AM, Ewase ASS, Soliman AM, Hussein SA. GC/MS-Based metabolomics profiling approach and determination of ameliorative effect of chiliadenus montanus extract towards CCl4 induced hepatotoxicity in albino rats. Egyptian Journal of Chemistry. 2021;64(7):3499-510. https://dx.doi.org/10.21608/ejchem.2021.65354.3401

[2] Ammar NM, Hassan HA, Abdallah HM, Afifi SM, Elgamal AM, Farrag ARH, et al. Protective effects of naringenin from Citrus sinensis (var. Valencia) peels against CCl4-induced hepatic and renal injuries in rats assessed by metabolomics, histological and biochemical analyses. Nutrients. 2022;14(4):841. https://doi.org/10.3390/nu14040841

[3] Otrubova O, Jerigova M, Halaszova S, Turecky L, Muchova J, Velic D. Rat liver intoxication with CCl4: biochemistry, histology, and mass spectrometry. Gen Physiol Biophys. 2018;37(5):527-35. doi: 10.4149/gpb_2018011

[4] EL Sayed HE, Morsy LE, Abo Emara TM, Galhom RA. Effect of carbon tetrachloride (CCl4) on liver in adult albino rats: histological study. The Egyptian Journal of Hospital Medicine. 2019;76(6):4254-61. https://dx.doi.org/10.21608/ejhm.2019.43804

[5] Iredale J, Benyon R, Pickering J, McCullen M, Northrop M, Pawley S, et al. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. The Journal of clinical investigation. 1998;102(3):538-49.

[6] Prieto JD, Alomar O, Agustí N, Battaglia D, Fanti P, Trotta V, et al. Does the plant defense priming compound β-aminobutyric acid affect the performance of Macrolophus pygmaeus when used to control Bemisia tabaci in tomato? Phytoparasitica. 2021;49:195-205. https://doi.org/10.1007/s12600-020-00850-3

[7] Al-Dulaimy EA, Jasim MA. Effect of non-protein amino acids β-amino butyric acid (BABA) on the intestinal G-bacterial community. 2022. https://doi.org/10.21203/rs.3.rs-1955225/v1

[8] Kim H, Uto T, Akagi T, Baba M, Akashi M. Amphiphilic poly (amino acid) nanoparticles induce size-dependent dendritic cell maturation. Advanced Functional Materials. 2010;20(22):3925-31. https://doi.org/10.1002/adfm.201000021

[9] Khorrami MB, Sadeghnia HR, Pasdar A, Ghayour-Mobarhan M, Riahi-Zanjani B, Hashemzadeh A, et al. Antioxidant and toxicity studies of biosynthesized cerium oxide nanoparticles in rats. International journal of nanomedicine. 2019:2915-26.

[10] Al-Dulaimy EAM, Jasim MA. Effect Of Non-Protein Amino Acid (BABA) On Gastrointestinal Tissues Of Male Rats And Some Negative Enterobacteria. Journal of Pharmaceutical Negative Results. 2022:219-25. DOI: 10.47750/pnr.2022.13.S02.31

[11] Lee D-Y, Kim E-H. Therapeutic effects of amino acids in liver diseases: current studies and future perspectives. Journal of Cancer Prevention. 2019;24(2):72.

[12] Mahmud JA, Hasanuzzaman M, Khan MIR, Nahar K, Fujita M. β-Aminobutyric acid pretreatment confers salt stress tolerance in Brassica napus L. by modulating reactive oxygen species metabolism and methylglyoxal detoxification. Plants. 2020;9(2):241. https://doi.org/10.3390/plants9020241

[13] Thamer IARH, Jasim MA. Anti-diabetic effect of β-amino Butyric Acid in Streptozotocin induced Rats. 2021.

[14] Hamad AI, Jasim MA. The effect of Beta-amino butyric acid (BABA) on Staphylococcus aureus bacteria and some interleukins. Journal of University of Anbar for Pure Science. 2024;18(1). DOI: 10.37652/juaps.2024.146193.1179

[15] Jasim, M.A. and Zgair, A.,. Hematological regulation using β-aminobutyric acid in staphylococcus aureus infected rats. Journal of Biotechnology Research Center. 2022; 16(2), pp.22-30. https://orcid.org/0000-0002-3247-5532

[16] Höhme S, Hengstler JG, Brulport M, Schäfer M, Bauer A, Gebhardt R, et al. Mathematical modelling of liver regeneration after intoxication with CCl4. Chemico-Biological Interactions. 2007;168(1):74-93.

[17] Uskoković-Marković S, Milenković M, Topić A, Kotur-Stevuljević J, Stefanović A, Antić-Stanković J. Protective effects of tungstophosphoric acid and sodium tungstate on chemically induced liver necrosis in wistar rats. J Pharm Pharm Sci. 2007;10(3):340-9.

[18] Lindqvist C, Slinde F, Majeed A, Bottai M, Wahlin S. Nutrition impact symptoms are related to malnutrition and quality of life–A cross-sectional study of patients with chronic liver disease. Clinical nutrition. 2020;39(6):1840-8. https://doi.org/10.1016/j.clnu.2019.07.024

[19] Badawy AA, Alamri AA, Hussein H-AA, Salem NF, Mashlawi AM, Kenawy SK, et al. Glycine betaine mitigates heavy metal toxicity in beta vulgaris (L.): an antioxidant-driven approach. Agronomy. 2024;14(4):797. https://doi.org/10.3390/agronomy14040797

[20] Amanpour P, Khodarahmi P, Salehipour M. Protective effects of vitamin E on cadmium-induced apoptosis in rat testes. Naunyn-Schmiedeberg's Archives of Pharmacology. 2020;393(3):349-58. https://doi.org/10.1007/s00210-019-01736-w

[21] Liu, J.Y., Chen, C.C., Wang, W.H., Hsu, J.D., Yang, M.Y. and Wang, C.J., 2006. The protective effects of Hibiscus sabdariffa extract on CCl4-induced liver fibrosis in rats. *Food and Chemical Toxicology*, *44*(3), pp.336-343. https://doi.org/10.1016/j.fct.2005.08.003

[22] Ben Hsouna A, Hfaiedh M, Ben Slima S, Romdhane WB, Akacha BB, Bouterra MT, et al. Antioxidant and hepatoprotective effects of novel heteropolysaccharide isolated from Lobularia maritima on CCl4-induced liver injury in rats. Food Science & Nutrition. 2022;10(7):2271-84. https://doi.org/10.1002/fsn3.2836

[23] Ugwu CE, Suru SM. Medicinal plants with hepatoprotective potentials against carbon tetrachloride-induced toxicity: a review. Egyptian Liver Journal. 2021;11:1-26. https://doi.org/10.1186/s43066-021-00161-0

[24] Amer MA, Othman AI, El-Missiry MA, Farag AA, Amer ME. Proanthocyanidins attenuated liver damage and suppressed fibrosis in CCl4-treated rats. Environmental Science and Pollution Research. 2022;29(60):91127-38. https://doi.org/10.1007/s11356-022-22051-7

[25] Chhimwal J, Sharma S, Kulurkar P, Patial V. Crocin attenuates CCl4-induced liver fibrosis via PPAR-γ mediated modulation of inflammation and fibrogenesis in rats. Human & Experimental Toxicology. 2020;39(12):1639-49. https://doi.org/10.1177/0960327120937048