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Anti-Hepatotoxic Effects of Aqueous and alcoholic Extracts of *Tribulus terrestris* Fruits and FertiPlus® on Albino Rats Exposed to Sulfasalazine

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Abstract

Tribulus terrestris is a medicinal plant traditionally used to treat liver disorders, infertility, and conditions associated with oxidative stress. It contains bioactive compounds such as saponins and flavonoids, which enhance its antioxidant and hepatoprotective properties.

Objective: The present study aimed to evaluate the anti-hepatotoxic effects of aqueous and alcoholic extracts of Tribulus terrestris fruits and a dietary supplement (FertiPlus®) on male albino rats susceptible to sulfasalazine-induced hepatotoxicity (SSZ).

Methods: Thirty-five male albino rats (180–230 g) were divided into five groups. In the first phase (4 weeks), four groups received oral sulfasalazine to induce hepatotoxicity, while one group served as a control. Hepatotoxicity was confirmed by necropsy and biochemical markers. In the second phase (also 4 weeks), the SSZ-exposed groups were treated with either the aqueous extract, the alcoholic extract, or FertiPlus®, while the other infected group was autopsied to detect liver damage (at day 30). Liver enzyme levels (AST and ALT), fat deposition, and liver histology were assessed. Analysis of variance (ANOVA) was used for statistical analysis ($P < 0.05$).

Results: SSZ administration led to a significant increase in AST and ALT levels and an increase in liver fat deposition, indicating liver damage. Treatment with Tribulus terrestris extracts and FertiPlus® resulted in significant improvements in liver enzyme levels and histological structure. The aqueous extract and FertiPlus® demonstrated the strongest hepatoprotective effects.

Conclusion: Tribulus terrestris extracts, particularly the aqueous extract, and the FertiPlus® supplement demonstrated protective effects against SSZ-induced hepatotoxicity in rats, indicating their therapeutic value in hepatoprotection.

تأثير المستخلص المائي والكحولي لثمار نبات التريبولوس تيريستريس والمكمل FertiPlus®

على الفئران البيضاء المعرضة للسلفاسالازين

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الخلاصة

تريبولوس تيريستريس نبات طبي يُستخدم تقليدياً لعلاج اضطرابات الكبد، والعقم، والحالات المرتبطة بالإجهاد التأكسدي. يحتوي على مركبات نشطة بيولوجياً مثل الصابونين والفلافونويد، مما يُعزز خصائصه المضادة للأكسدة والحماية للكبد. هدفت الدراسة الحالية إلى تقييم التأثيرات المضادة للسمية الكبدية للمستخلصات المائية والكحولية لثمار تريبولوس تيريستريس ومكمل غذائي (FertiPlus®) على ذكور الفئران البيضاء المعرضة لسمية الكبد المُحفزة بعقار السلفاسالازين (SSZ). قُسّم خمسة وثلاثون فأراً أبيض ذكراً (180-230 غرام) إلى خمس مجموعات. في المرحلة الأولى (4 أسابيع)، تلقت أربع مجموعات سلفاسالازين عن طريق الفم لتحفيز السمية الكبدية، بينما كانت مجموعة واحدة بمثابة مجموعة ضابطة. تم تأكيد السمية الكبدية من خلال التشريح والعلامات الكيميائية الحيوية. في المرحلة الثانية (4 أسابيع ايضا)، عولجت المجموعات المعرضة لـ SSZ إما بالمستخلص المائي، أو المستخلص الكحولي، أو FertiPlus®، بينما المجموعة المصابة الاخرى تم تشريحها للتحري عن الاصابة (عند يوم 30). تم تقييم مستويات إنزيمات الكبد (AST وALT)، وترسب الدهون، ونسيج الكبد. استُخدم تحليل التباين (ANOVA) للتحليل الإحصائي ($P < 0.05$). أدى تناول SSZ إلى ارتفاع ملحوظ في مستويات AST وALT وزيادة في ترسب الدهون في الكبد، مما يشير إلى تلف الكبد. أدى العلاج بمستخلصات تريبولوس تيريستريس وFertiPlus® إلى تحسينات ملحوظة في مستويات إنزيمات الكبد والبنية النسيجية. أظهر المستخلص المائي FertiPlus® أقوى التأثيرات الوقائية للكبد. أظهرت مستخلصات تريبولوس تيريستريس، وخاصةً المستخلص المائي، والمكمل FertiPlus® تأثيرات وقائية ضد سمية الكبد.

Introduction

Sulfasalazine (SSZ) is a medication widely used to treat several inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, and rheumatoid arthritis (RA). [1,2]. This medication lessens intestinal inflammation, but it has a lot of detrimental side effects on different organs. The liver is one of the vital organs that is impacted by sulfasalazine use [3]. Increased levels of liver enzymes, which signify liver toxicity or destruction to liver cells, are one way that it affects the liver [4]. Furthermore, this medication may cause fatty liver, a condition in which the liver accumulates fat. Fatty liver disease may arise from this syndrome [5], it ultimately results in liver dysfunction and raises health risks [6]. With the proliferation of research studies on the side effects of chemotherapy, there is an urgent need to discover and develop natural herbal therapeutic alternatives that reduce the side effects of these drugs on the animal body [7]. Plant extracts have gained significant attention in the field of treatments, especially the fruits of *T. terrestris* [8]. Many recent studies have shown that this plant contains natural chemical compounds such as alkaloids, flavonoids, tannins, and terpenoids, which are of great importance, especially since they are considered among the most important antioxidants. In addition, they are also anti-inflammatory agents resulting from certain factors [9]. Therefore, they are considered of great importance in various treatments for the body, especially liver diseases and protecting the liver from damage and toxic effects resulting from the negative effects of toxic drugs taken by the body [10]. One of the most prominent plants that have shown unique results in this field is *T. terrestris*, which contains effective compounds, especially in the fruits of the plant, which have antioxidant properties and also protect the liver from chemical toxins that affect it [11]. Many studies have indicated that this plant helps reduce liver inflammation and improve liver enzyme levels, especially AST and ALT, making it of great importance in natural treatments for the negative effects of drugs, especially in reducing the toxic side effects of sulfasalazine [12]. The elevation of liver enzymes, particularly aspartate and alanine transaminases (AST and ALT), is a well-

established indicator of hepatic injury across various conditions, including drug-induced toxicity [13,14] It is also important to mention the role of nutritional supplements, as they play an important role in enhancing liver function, treating the liver, and protecting it from the negative effects of medications [15]. These foods are popularly known as foods with specific health uses (FOSHU) [16]. They contain important compounds, for example, they contain vitamin C, arginine, and omega-3, which are among the most important basic ingredients in nutritional supplements that contribute to protecting and repairing the liver from damage caused by nutrition or medications [17]. Vitamin C acts as a potent antioxidant in the human body [18,19]. Arginine is an essential amino acid that improves circulatory and liver function. It also contributes to the production of nitric oxide, which improves blood flow and reduces liver toxicity caused by medication. It is also an antioxidant [20]. In addition, nutritional supplements containing essential fatty acids such as omega-3 and vitamin E help reduce liver inflammation, strengthen the liver's defense mechanism, and regulate its function [21]. The objectives of the study were to explore the protective role of plant extracts, such as *T. terrestris*, against the adverse effects of sulfasalazine on the liver, to evaluate the effectiveness of FertiPlus®, such as zinc, vitamin C, and arginine, in reducing oxidative damage caused by sulfasalazine and explore safer natural therapeutic alternatives to reduce the side effects of chemical drugs on the liver.

Materials & Methods

Plant Collection

Pruit samples were collected on September 12, 2024, when the fruits were in the ripening stage. They were picked from agricultural fields in the Al-Zwiya area of Baiji District and classified by Professor Dr. Ali Al-Moussawi in the herbarium of the College of Science - University of Baghdad under the scientific name *Tribulus terrestris*, belonging to the Zygophyllaceae family. The fruits were moist and washed with water to remove dust and impurities. They were then placed on a cloth in a cool, dry place away from sunlight for two to three weeks. After ensuring complete dryness, the samples were ground using an electric machine, and the powder was stored in a tightly sealed glass container away from light, heat, and humidity until ready to use. The fruits were ground using an electric grinder for five minutes to obtain a fine powder ready for extraction. Then, 50 grams of dry powder was weighed and placed in a 1000 ml glass beaker, to which 500 ml of distilled water was added. The contents were mixed using a heating device with a magnetic stirrer for 20 minutes at 40°C. The mixture was then left on a shaker for 24 hours. The mixture was then filtered through several layers of gauze, and the insoluble materials were separated using a centrifuge. The resulting extract was placed in 250 ml glass dishes, which were then placed in an electric oven at 37°C to obtain the crude aqueous extract. The process was repeated several times to obtain a sufficient quantity of the extract. It was then placed in a tightly sealed glass container, isolated from light, and stored in the refrigerator until used for animal dosing [22].

Design and Experimental Animals

This experimental study was carried out at Tikrit University Animal House in Salahaddin, from September 30, 2024, to November 30, 2024 (over a span of 8 weeks). Thirty-five adult male rats *Rattus norvegicus* (*Wistar strain*) in good health, at the beginning of the trial weighed between 180 and 220 grams and were roughly 8–10 weeks old. These animals were sourced from the College of Veterinary Medicine's Animal House at Tikrit University in the Salahaddin Governorate. Every rat group was put separately in a transparent, Cages made of sterilized plastic intended for experimentation, with measurements of 13 x 28 x 46 cm, and with similar weights to guarantee a steady dosing. The cages were kept in a laboratory under strict controls, with 50–60% humidity set. Before the trial began, the animals were given a week to acclimate. The lighting system ran on a 12-hour light/dark cycle, and the temperature was maintained at 25°C ± 2°C, and adequate ventilation was guaranteed. Water and diet prepared in accordance with the guidelines were given to the rats. 35 rats divided into 5 groups (7 in each group) for the two equal periods of the

experiment, each lasting 4 weeks, each with seven rats, as seen below:

Phase One (4-week induction phase)

Group One (Healthy Control Group): For the full 8 weeks, this group was fed normal food. The remaining Four groups were induced with Sulfasalazine, administered orally at a precisely measured amount of 150 mg/kg body weight distilled water used as a solvent for the drug [23]. Animals from the second group were dissected at the conclusion of the 4-week treatment period to assess histological damage, liver function, and biochemical indicators of hepatic injury.

Phase Two (4-week treatment phase)

Group One **(**Healthy Control Group**)**: Adherence to the same natural diet was maintained. Group Two **(**Infected Control Group) The second group (infected mice) was dissected and blood and liver tissue samples were taken at the end of 30 days. Group Three (SSZ-treated): To evaluate its protective effects against SSZ-induced liver damage, they were given an aqueous extract of *T. terrestris* fruit at a dose of 13 mg/kg body weight every day for 4 weeks [24].Group Four (SSZ-treated): Treated for the same amount of time and with an alcoholic extract of *T. terrestris* fruit to investigate its potential to reduce SSZ-induced liver toxicity.Group Five (SSZ-treated): Animals were given the supplement daily at a dose of 2.5 mg/kg body weight for 30 days to evaluate its potential in reducing SSZ-induced liver damage as well as in improving liver function.

Drugs Utilized in the Study

Sulfasalazine: Rats were given a dose of 150 mg/kg/day of sulfasalazine to evaluate the known side effects of sulfasalazine on liver function, liver enzymes, and histological changes.

FertiPlus® (Basic Nutrition Ltd., UK): in the current study, this supplement was administered orally at a dose of 2.5 mg/kg/day. This formulation is known for its antioxidant and hepatoprotective properties. The composition of the dose is shown in Table 1.

Collection of Blood Samples

Prior to dissection, I fasted the animals for 12 hours and then euthanized them using an intraperitoneal overdose of Ketamine (100 mg/kg) and Xylazine (10 mg/kg), following internationally accepted ethical guidelines. After confirming loss of reflexes, and underwent cardiac puncture to collect 5 ml blood samples. The blood was then centrifuged at 3000 rpm for 10 minutes, and the serum was separated using a micropipette. The serum samples were stored at -80°C in Eppendorf tubes until further biochemical analyses: Liver Enzymes (AST, ALT) and Lipid Profile parameters triglycerides (TG), total cholesterol (TC), HDL, LDL, VLDL. Were measured using commercial diagnostic kits based on colorimetric assay methods, according to the manufacturer's instructions. The readings were performed using the Humalyzer Primus® spectrometer (Human Diagnostics Worldwide, Germany).

Histopathological examination

Rats were dissected after being fixed on a dissection board, and their livers were carefully removed and placed in normal saline (0.9%). After removing the blood, the livers were then placed in 10% formalin for a period of time (48 hours), after which we performed histological analysis of the liver tissue. Thin sections of liver tissue were prepared and stained with hematoxylin and eosin (H&E). According to [25], the stained sections were examined under a light microscope to evaluate the structural composition of the liver tissue, including the integrity and morphology of hepatocytes, the liver structure, and the presence of any histopathological changes such as necrosis, inflammation, and degeneration.

Results

The results of the current study, as shown in (Fig. 1), showed statistically significant differences between the groups ($P \leq 0.05$). The infected control group showed a significant decrease in AST levels (106.8±6.42) U/L compared to the healthy control group (134.0±7.38) U/L. As for the treated groups, the group treated with the alcoholic extract (122.6±9.42) U/L showed a significant

increase compared to the infected control group, while the group treated with the nutritional supplement (82.6±9.21) U/L showed a significant decrease compared to the infected control group, while the group treated with the aqueous extract (104.2±8.76) U/L did not show any significant change compared to the infected control group. The results of the current study, as shown in (Fig. 2), showed no statistically significant differences (P>0.05) in the ALT level between the infected control group (56.2±7.64) U/L compared to the healthy control group (52.0±5.34) U/L, while all treatment groups, the group treated with the aqueous extract (46.8±5.12) U/L, the group treated with the alcoholic extract (41.0±7.31) U/L, and the group treated with the nutritional supplement (35.2±4.21) U/L, showed a significant decrease compared to the infected control group. While the test result (Fig. 3) showed a significant decrease (P≤0.05) in the level of triglycerides in the infected control group (42.1±7.3) compared to the healthy control group (86.4±8.9), while the groups treated with the aqueous extract of the plant (92.1±9.5) and the group treated with the nutritional supplement (148.8±9.7) recorded a significant increase compared to the infected control group, while the alcoholic extract (48.6±6.9) did not show a statistically significant change compared to the infected control group. The results of the cholesterol level study, as shown in (Fig. 4), showed that there was no significant difference at the probability level (P≤0.05) in the cholesterol level in the infected control group (58.8±7.8) compared to the healthy control group (57.4±8.1), while no significant change was observed except in the group treated with the alcoholic extract (78.2±9.3), where a significant increase was observed with statistical significance compared to the infected control group. While the results of the study of HDL levels, as shown in (Fig. 5), showed a significant increase (P≤0.05) in HDL levels in the infected control group (45.8±7.8) compared to the healthy control group (44.2±6.4), while the group treated with the alcoholic extract showed a significant increase (60.8±9.7) compared to the infected control group, while the group treated with the nutritional supplement (60.8±9.7) recorded a significant decrease compared to the infected control group, while the group treated with the aqueous extract (48.8±8.7) did not show any statistical significance compared to the infected control group. While the results of the LDL study, as shown in (Fig. 6), showed statistically significant differences between the groups (P≤0.05). The infected control group showed a significant increase in LDL level (4.66±1.19) compared to the healthy control group (2.02±0.90), while the group treated with the aqueous extract (2.14±0.07) recorded a significant decrease compared to the healthy control group, while the group treated with the alcoholic extract (4.61±1.89) did not record any statistical significance compared to the infected control group, while the group treated with nutritional supplements (13.56±2.23) showed a significant increase compared to the infected control group. The results of the VLDL study, as shown in (Fig. 7), showed statistically significant differences between groups (P≤0.05), as the infected control group showed a significant decrease in the VLDL level (8.4±1.5) compared to the healthy control group (17.5±2.5), while the treated groups, the aqueous extract treatment group (18.8±3.7), the alcoholic extract treatment group (11.2±3.3), and the nutritional supplement treatment group(32.1±4.1), showed a significant increase compared to the infected control group.

Table 1: shows the ingredients of FertiPlus®

Component	Amount per Capsule (mg)
Maca	250
Horny Goat Weed	500
Semen Cuscutae (Dodder Seed)	180
L-Arginine HCl	250
Vitamin C	120
Zinc	14

Table 2: Liver enzymes (AST and ALT) and Lipid Profile in serum. Values represent the mean ± standard deviation. Number of rats (7) in each group. Different letters indicate significant differences at P≤0.05.

Groups	AST (mIU/ml)	ALT (mIU/ml)	TG (Mg/d)	TC (Mg/d)	HDL (Mg/d)	LDL (Mg/d)	VLDL (Mg/d)
Healthy control (60 days)	134.0±7.4 a	52.0±5.3 a	86.4±8.9 b	57.4±8.1 b	44.2±6.4 bc	2.02±0.9 c	17.5±2.5 b
Infected control (30 days)	106.8±6.4 c	56.2±7.6 a	42.1±7.3 c	58.8±7.8 b	45.8±7.8 b	4.66±1.2 b	8.4±1.5 d
Infected (30 days) Then Treated with aqueous extract (30days)	104.2±8.8 c	46.8±5.1 b	92.1±9.5 b	61.2±3.1 b	48.8±8.7 b	2.14±0.1 c	18.8±3.7 b
Infected (30 days) Then Treated with alcoholic extract (30 days)	122.6±9.4 b	41.0±7.3 b	48.6±6.9 c	78.2±9.3 a	60.8±9.7 a	4.61±1.9 b	11.2±3.3 c
Infected (30 days) Then Treated with nutritional supplement (30 days)	82.6±9.2 d	35.2±4.2 c	148.8±9.7 a	56.1±9.1 b	39.5±6.5 c	13.56±2.2 a	32.1±4.1 a
P-Value	0.0002**	0.0008**	0.00002**	0.025*	0.025*	0.0004**	0.0008**

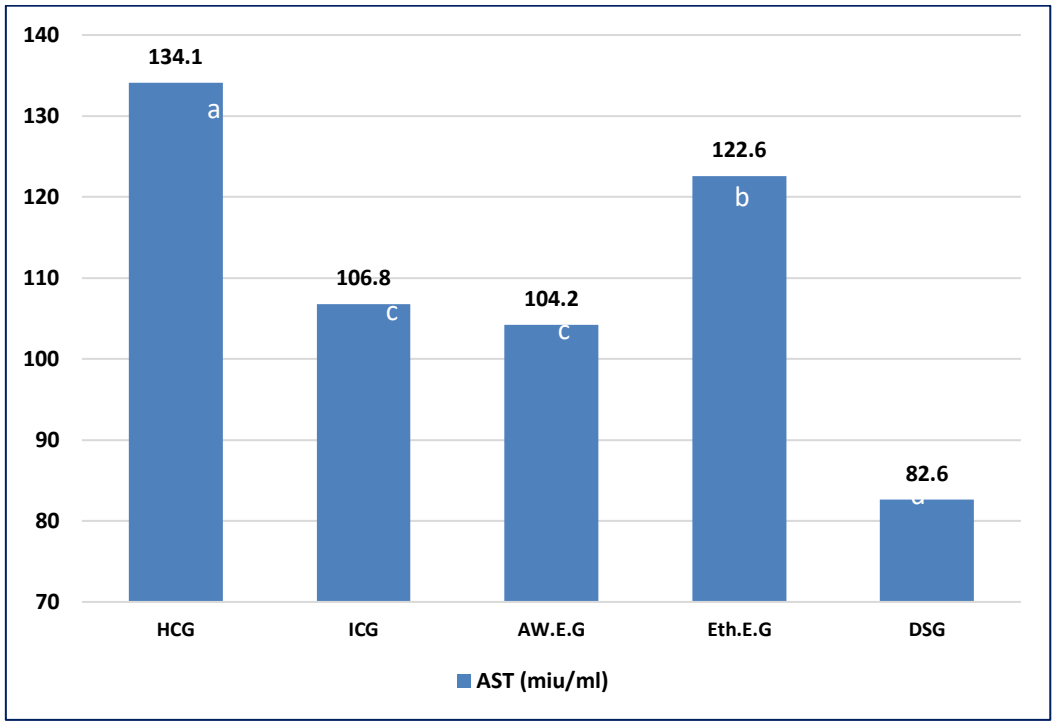

Figure 1: A significant change appeared at (P≤0.05) between the different groups, as there was a noticeable difference between the control group and the different treatments. Healthy Control Group (HCG), Infected Control Group (ICG), Aqueous Extract Treatment (AW.E.G), Ethanol Extract Treatment (Eth.E.G), Dietary Supplement (DSG). Different letters mean significant differences at the P≤0.05 level.

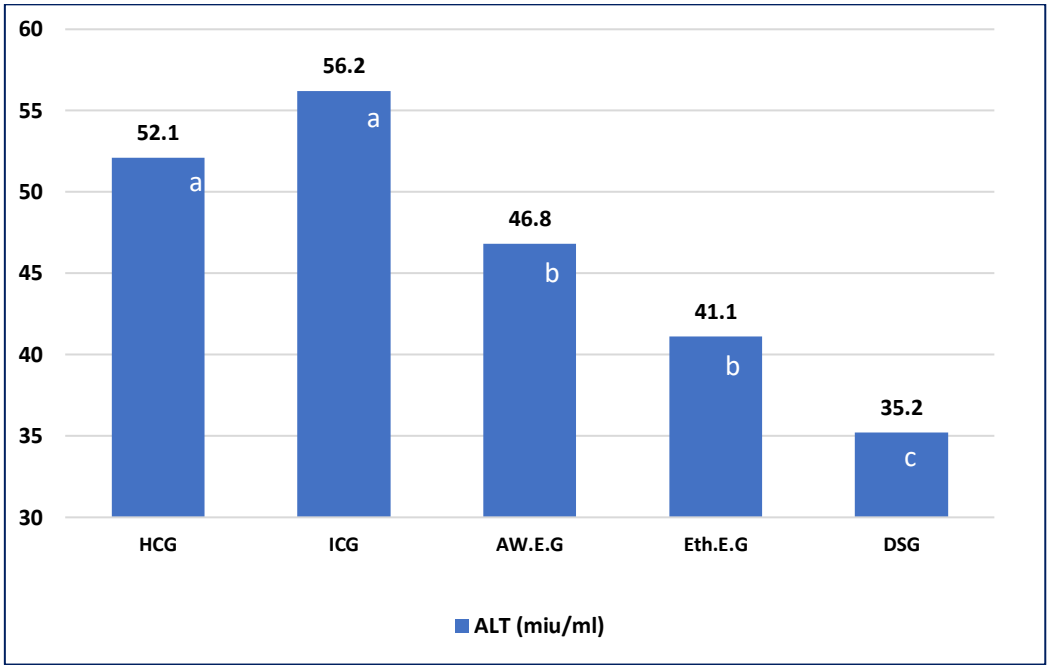

Figure 2: A significant change was observed at (P≤0.05) as there was a significant decrease in the level of ALT enzyme in the treated groups compared to the control group, the greatest of which was in the nutritional supplement.

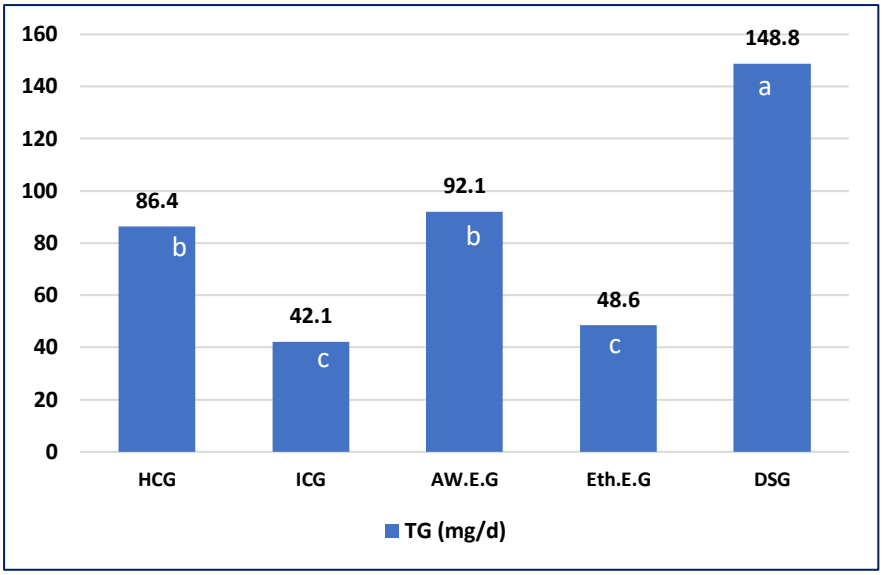

Figure 3: The results show a significant change at (P≤0.05), as there was a significant increase in the TG level in the group treated with the nutritional supplement compared to the rest of the groups. Different letters mean significant differences at P≤0.05 level.

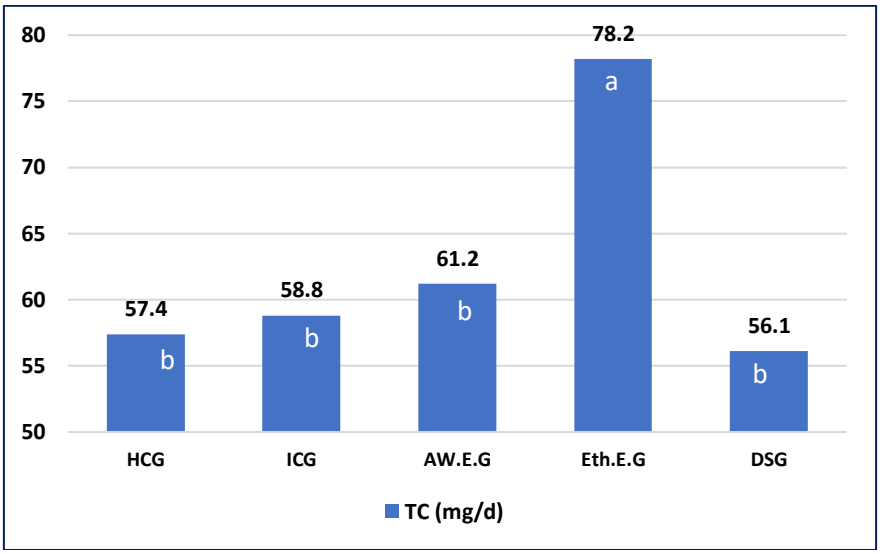

Figure 4: A significant change was observed at (P≤0.05), as there was a significant increase in the level of TC in the group treated with alcoholic extract compared to the rest of the groups. Different letters mean significant differences at the P≤0.05 level.

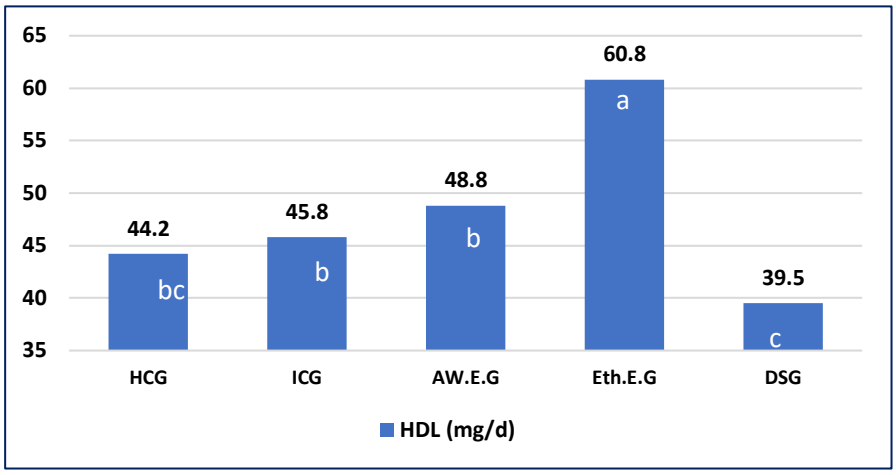

Figure 5: The results showed a significant change at (P≤0.05), as there was a significant increase in the level of HDL in the group treated with the alcoholic extract compared to the rest of the groups. Different letters mean significant differences at the P≤0.05 level.

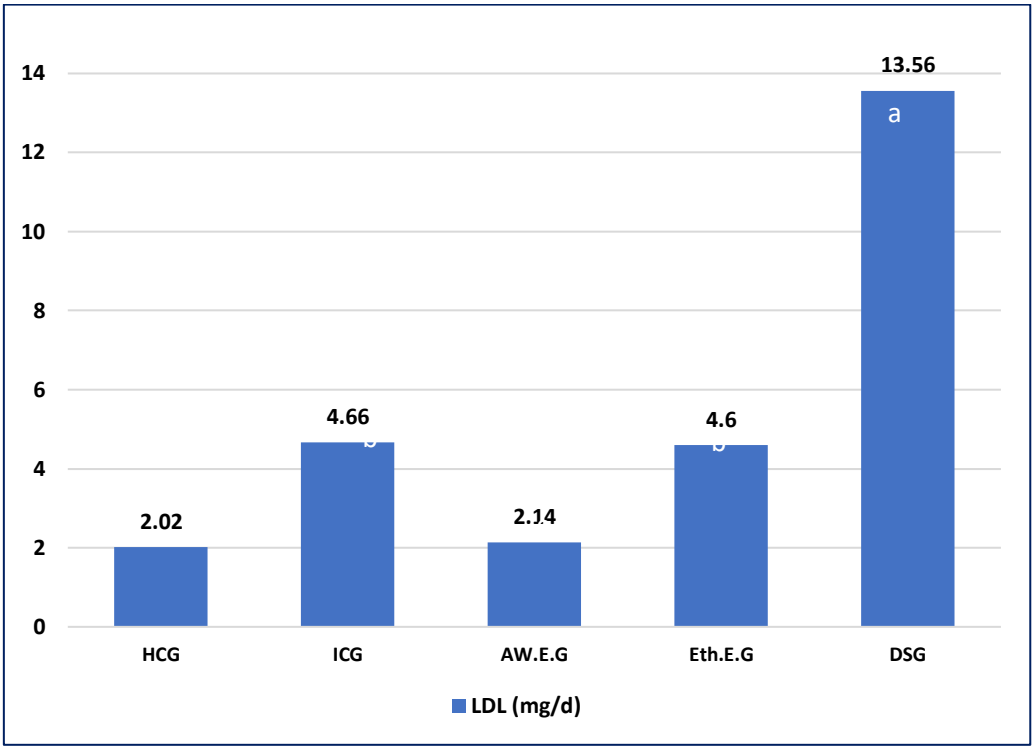

Figure 6: A significant change was observed at (P≤0.05), as there was a notable increase in the LDL level in the group treated with the nutritional supplement compared to the control and other treatment groups. Different letters mean significant differences at the P≤0.05 level.

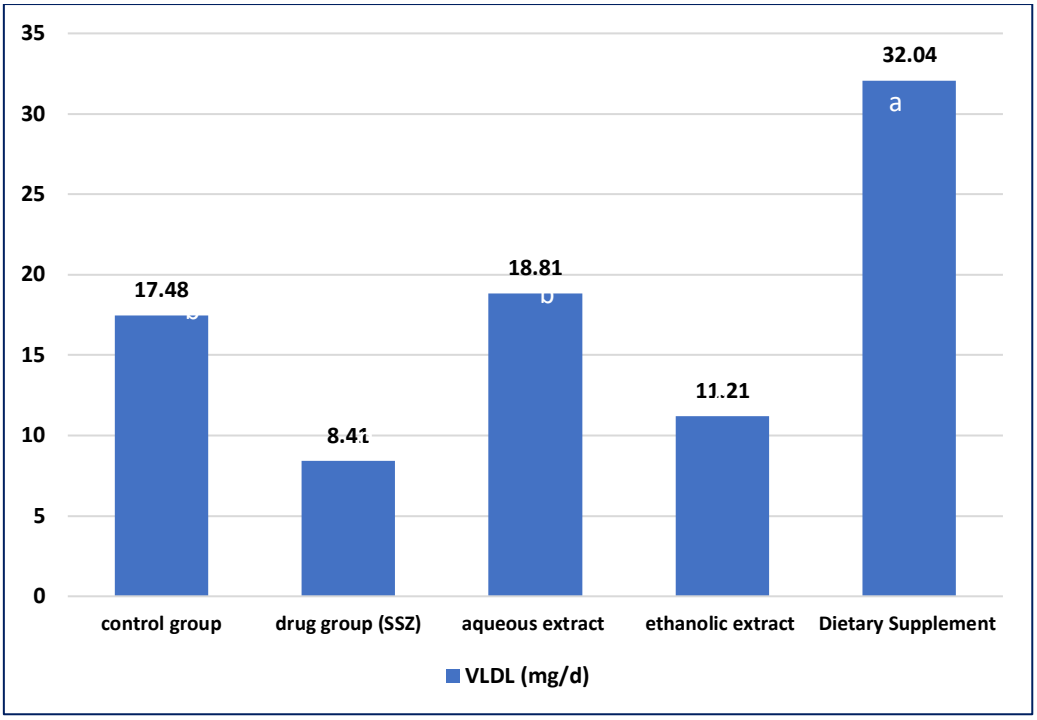

Figure 7: The results showed a significant difference at (P≤0.05), as there was a significant increase in the level of VLDL in the group treated with the nutritional supplement compared to the other groups. Different letters mean significant differences at the P≤0.05 level.

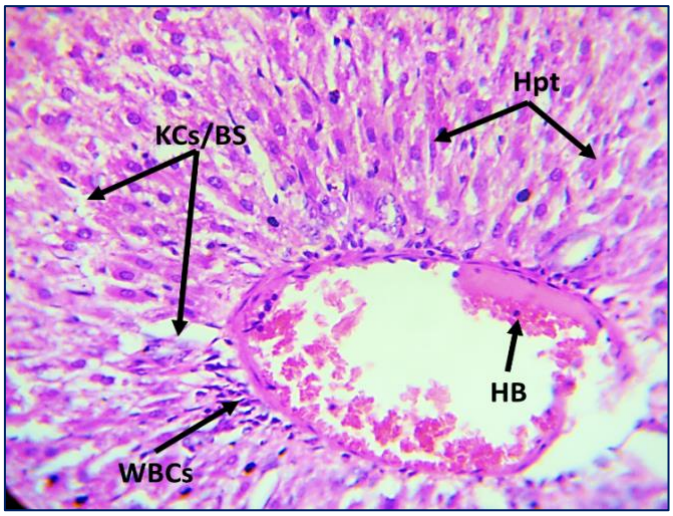

Figure 8: Liver tissue showing a wide-bore central vein containing hemolyzed blood (HB), with white blood cells (WBCs) around the vein, radial rows of hepatocytes (Hpt), blood sinusoids (BS) containing numerous Kupffer cells (KCs), (H&E × 40).

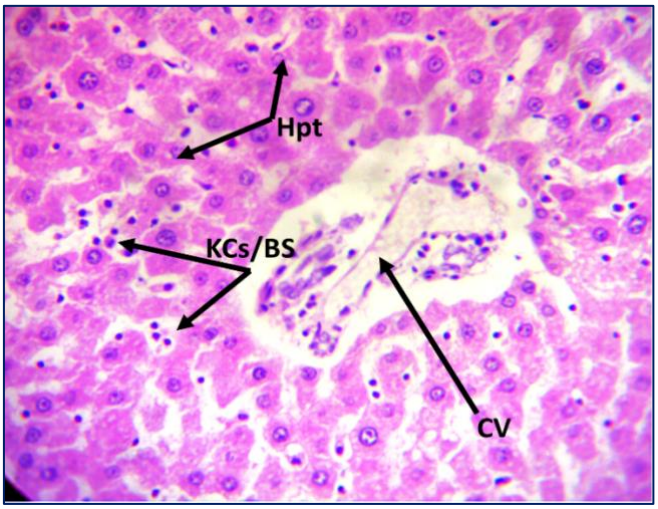

Figure 9: Liver tissue showing a central vein (CV) with sloughing of the basement membrane and its overlying endothelial cells, accompanied by infiltration of white blood cells. Polygonal hepatocyte (Hpt) rows, wide-lumened blood sinusoids (BS) filled with Kupffer cells (KCs) are also observed. (H&E, × 40).

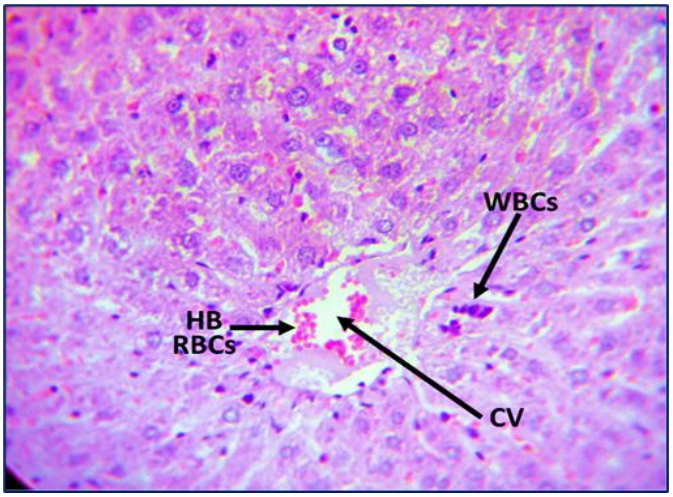

Figure 10: Liver tissue showing a central vein (CV) containing hemolyzed blood (HB) and red blood cells (RBCs), along with aggregates of white blood cells (WBCs). Hypertrophy of hepatocytes, narrow sinusoids with Kupffer cells, and areas of normal hepatocytes are also observed. (H&E, × 40).

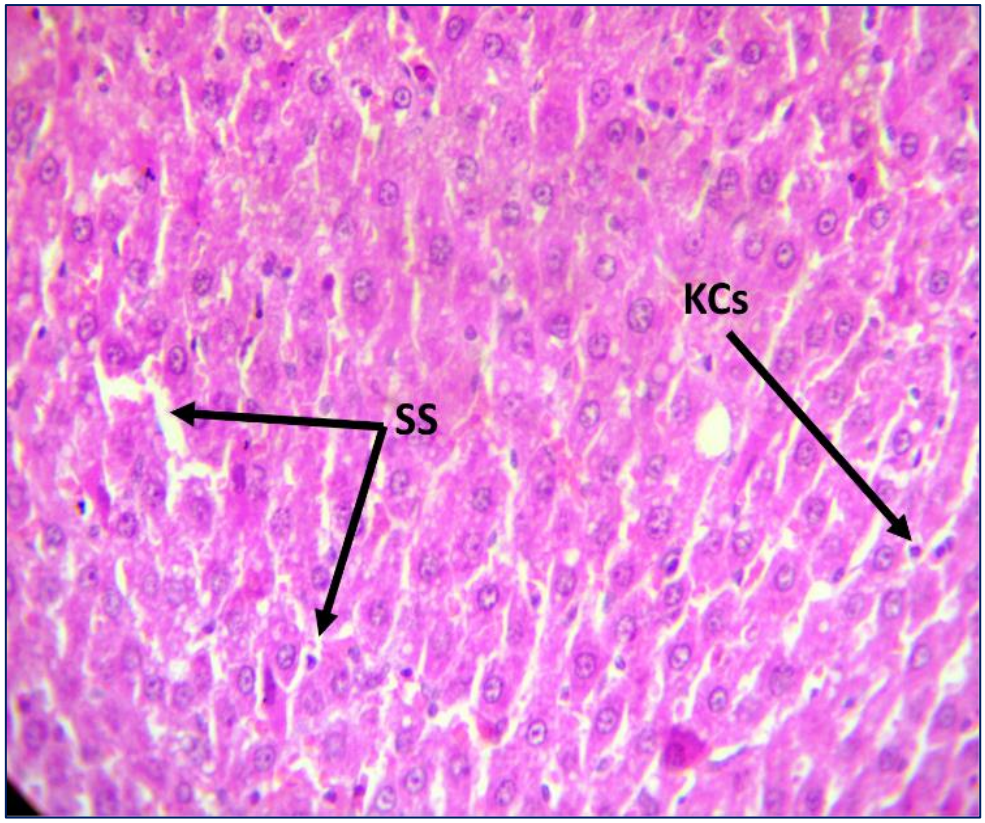

Figure 11: Liver tissue, close-packed rows of hepatocytes with a spherical nucleus, narrow sinusoids (SS) with some Kupffer cells (KCs). (H&E, × 40).

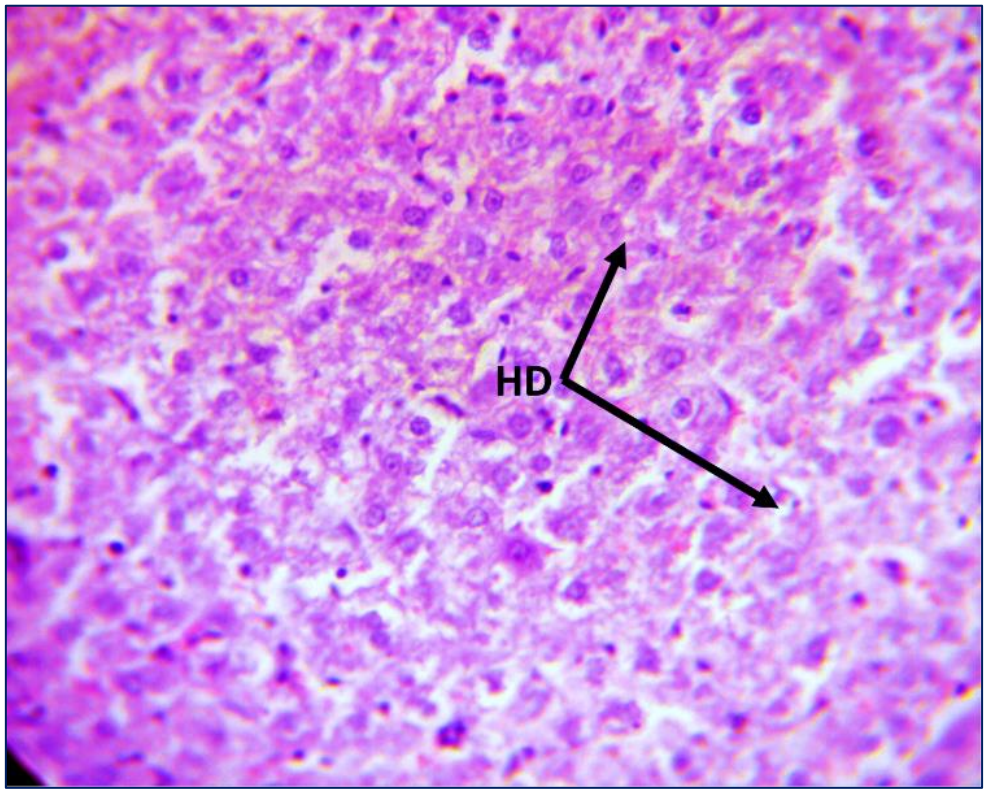

Figure 12: Liver tissue showing hepatocyte degeneration (HD), normal hepatocytes, and Kupffer cells, in blood sinusoids. (H&E, × 40).

Discussion

The results of the current study showed statistically significant differences in the levels of the liver enzymes AST and ALT between the groups. In the first phase, the infected control group exposed to sulfasalazine showed a significant decrease in AST compared to the healthy control group, reflecting damage to hepatic cells and the loss of their ability to secrete the enzyme as a result of the toxic drug. A study [26] also confirmed the effect of sulfasalazine on liver damage and enzyme disturbances. In the second phase, the group treated with the aqueous extract of *Tribulus terrestris* showed a decrease in ALT without a significant improvement in AST, indicating a partial effect in restoring liver function. This was supported by a study [27] on the effect of the aqueous extract in reducing liver enzymes. The group treated with the alcoholic extract showed a significant increase in AST and a decrease in ALT compared to the infected control group, reflecting an effective response in restoring liver tissue. These results are supported by a study [28] that demonstrated the effectiveness of the alcoholic extract in improving biomarkers. The FertilitePlus supplement group showed lower levels of ALT and AST, indicating a strong protective effect, consistent with the study [29]. Despite the significant reduction in enzymes, the histological improvements were less pronounced compared to the plant extracts.

The results of the current study showed significant changes in lipid profiles following exposure to sulfasalazine, as well as after treatment with the three treatments. In the first phase (infection), the infected control group showed a significant decrease in triglyceride levels (TRIs) compared to the healthy group, indicating a negative effect of the drug on lipid metabolism. Sulfasalazine is known to affect lipid absorption and formation. This is possibly due to its effect on the intestinal mucosa or its interactions with bile acids. A study [30] also showed that sulfasalazine can induce changes in the absorption of fats and proteins. In the treatment phase, the group treated with the aqueous extract of *Tribulus terrestris* showed a significant improvement in triglyceride levels, even exceeding the level of the healthy control group. This reflects the effectiveness of this extract in regulating lipids by improving hepatic lipase activity and increasing fatty acid utilization.

A study [31] demonstrated the positive effect of the aqueous extract on lipid metabolism in diabetic rats. While the alcoholic extract did not significantly alter triglycerides, this may be due to differences in the chemical composition of the extract and its absorption pathways, consistent with a study [32]. The FertilitePlus supplement group showed a significant increase in triglyceride levels, suggesting that the supplement may contain lipid-stimulating compounds such as zinc or plant hormones. This finding contradicts some studies that have indicated neutral effects of supplements on lipids, such as [33]. Regarding total cholesterol (CHO), the affected control group did not show a significant difference compared to the healthy control group, indicating that these parameters are not directly affected by sulfasalazine. However, the alcoholic extract group showed a significant increase in total cholesterol, which is interpreted as a result of the activation of cholesterol synthesis by alcoholic components such as plant steroids, a finding supported by this study [34]. However, the nutritional supplement and the aqueous extract did not significantly alter this parameter. As for high-density lipoprotein (HDL), the alcohol extract group recorded a significant increase, indicating the effectiveness of this extract in raising good cholesterol by stimulating enzymes such as lecithin–cholesterol acyltransferase (LCAT), which recycle cholesterol to the liver. This result is consistent with the study [35]. Meanwhile, the dietary supplement group showed a significant decrease, indicating its weak effectiveness in this regard. This result contradicts the findings of the study [36]. Low-density lipoprotein (LDL) levels increased significantly in the affected control group, reflecting the onset of a metabolic disorder resulting from the effect of sulfasalazine on lipid metabolism. The aqueous extract showed a significant decrease in LDL, confirming its ability to improve the LDL/HDL ratio, which is consistent with the results of the study [37]. In contrast, the alcoholic extract did not show a

significant improvement, while the dietary supplement caused a severe negative increase in LDL, raising doubts about its metabolic safety.

As for VLDL levels, the sulfasalazine group showed a significant decrease, while the supplement group recorded a significant increase, indicating an imbalance between cholesterol and triglycerides. The other treatment groups showed varying improvements in this indicator. Liver histology results showed clear toxic effects on liver tissue in the group exposed to sulfasalazine (SSZ) [25]. Dilation of the central vein and damage to hepatocytes were observed [38], along with an increased number of Kupffer cells in the blood sinusoids [39]. In contrast, treatment with the aqueous extract of *Tribulus terrestris* fruits showed a clear improvement in liver tissue, as hepatocyte arrangement became more regular with some improvement in nuclei organization, despite some cytoplasmic degeneration [40].

Treatment with the alcoholic extract showed greater improvement in tissue condition, with hepatocytes being more organized with spherical nuclei, and fewer Kupffer cells compared to the aqueous extract-treated group [41], supporting the effectiveness of the alcoholic extract in reducing toxic effects [11]. In comparison, the effectiveness of the FertilitePlus nutritional supplement was lower compared to plant extracts, with greater liver tissue damage observed compared to plant extracts [13].

Conclusions: These results highlight the study results show that plant extracts and nutritional supplements have positive effects in reducing sulfasalazine-induced hepatotoxicity, as these treatments contribute to improving the biochemical and functions of the liver. and in improving liver tissue condition compared to nutritional supplements after liver toxicity.

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