Experimental Study of the Protective Effects of Capparis Spinosa Aqueous Extract on Carbon Tetrachloride-Induced Toxicity in Female Rabbits

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Abstract: This study investigates the protective potential of an aqueous extract of Capparis spinosa against carbon tetrachloride (CCl_4)-induced toxicity and its effects on selected hematological and biochemical parameters in female rabbits. Twelve rabbits were randomly assigned into three groups (n = 4 per group): a control group receiving a standard diet, and two experimental groups administered CCl_4 at doses of 0.2 mL/kg and 0.3 mL/kg, respectively. After a three-week exposure period, the experimental groups were subsequently treated with Capparis spinosa aqueous extract for three weeks at doses of 2 mL and 3 mL, respectively. Hematological indices (RBC, WBC, Hb, lymphocytes, platelets) and biochemical markers (glucose, cholesterol, triglycerides, total protein, urea, ALT, AST, and ALP) were evaluated. The results indicated that CCl_4 exposure induced significant alterations in both hematological and biochemical parameters, including reductions in RBC, Hb, WBC, and lymphocyte counts, as well as elevated liver enzyme activity. Treatment with the plant extract showed partial restorative effects, improving RBC, platelet counts, and reducing ALT levels. However, disturbances in lipid and protein metabolism persisted. Overall, the findings suggest that Capparis spinosa exhibits partial hepatoprotective and hematopoietic effects against CCl_4-induced toxicity, supporting its potential as a natural antioxidant-based therapeutic agent.

1 INTRODUCTION

Ever since the beginning of human history, people have utilized herbs and medicinal plants both naturally and via experimentation to treat a wide range of illnesses and symptoms. Despite scientific and technological advances in the creation of chemical medicines, which can quickly and effectively treat a wide range of illnesses, the therapeutic advantages of chemical drugs are not without risk. Abuse and haphazard use of these medications have raised the level of microbial resistance, giving rise to strains of bacteria that are highly resistant to them. A growing number of people are interested in using medicinal plants as a good substitute for conventional therapies because of these and other reasons. [1], [2]. Throughout history, medicinal plants have been utilized for food and medicinal purposes, making them some of the most significant and ancient plants known to humans [3], [4]. Since medicinal plants are a basic source of health for humans [5], [6] and the World Health Organization estimates that around 80% of the population in most countries depends on these plants for vital medical treatment, their significance in both medical and economic terms has become clear. Herbal therapy is prevalent today despite the existence of contemporary medicine. Numerous traditional cultures emphasize the curative and preventive benefits of herbal remedies, alongside with additional advantages such as cost-effectiveness and accessibility [2], [7]. One chemical compound that is generated industrially is carbon tetrachloride. An odorous, clear liquid quickly evaporates and is a member of the alkyl halide class of organic compounds [8]. Its chemical characteristics, including the existence of a carbon atom, sluggish molecular interactions, and separate reactions, are what classify it in this category [9]. Henri Victor Regnault, a French chemist, first synthesized it in 1839 by the reaction of chlorine with chloroform. It was synthesized in 1950 by heating carbon disulfide to temperatures ranging from 103 to 150 degrees Celsius in the presence of chlorine. It is currently mostly made from methane gas. Capparis spinosaon is a bluish-green perennial evergreen plant belonging to the Capparidaceae family. The plant is known by many names in the Arab world, including "Kabar," "Qabar," "Capparis," "Cotton," and "Mountain Pepper," among others. It has sprawling branches, thick leaves with spiny stipules, large flowers, and fleshy fruits resembling pears, supported by a long stalk. The size of the leaves and flowers varies among species, with about 250 types known [10]. Given the significance of this subject, an investigation was carried out to determine how injections of carbon tetrachloride affected specific blood parameters. Additionally, an assessment was made of the protective effects of Capparis spinosaon's aqueous extract on the alterations in blood parameters brought about by carbon tetrachloride injections.

2 MATERIALS AND METHODS

The study was carried out at the College of Basic Education / Haditha, University of Anbar, at the Animal House of the Department of General Sciences. Twelve female bunnies, six to seven months old, were employed in this study. Every rabbit was maintained under controlled environmental conditions and in good health. After being fed their designated diet, the animals were randomly divided into three groups ($n = 4$ per group): Group 1 (Control) received only standard feed; Group 2 received feed supplemented with 0.2 mg/kg of carbon tetrachloride (CCl_4); and Group 3 received 0.3 mg/kg of CCl_4. The exposure period lasted three weeks.

After the induction phase, Capparis spinosa aqueous extract was administered to Groups 2 and 3 twice daily for an additional three weeks (2 ml for Group 2 and 3 ml for Group 3). At the end of the experiment, blood samples were collected via cardiac puncture. Serum and whole blood were separated for hematological and biochemical analysis.

Red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), lymphocytes (LYM), and platelet count (PLT) were measured, along with biochemical parameters including cholesterol, triglycerides, glucose, urea, total protein, and liver enzymes (ALT, AST, ALP).

The plant material was collected from desert regions of Haditha, Anah, and Al-Baghdadi. Mature fruits were dried under sunlight, cleaned, and ground into powder. Aqueous extract was prepared by dissolving 125 g of plant material in distilled water, followed by magnetic stirring for 4–6 hours and filtration over 24 hours. The filtrate was stored under appropriate conditions until use.

For statistical analysis, data were processed using SPSS Statistics version 26. Results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine statistical significance among groups, with $p < 0.05$ considered significant.

3 RESULTS AND DISCUSSION

Figure 1 illustrates the effect of the toxic chemical carbon tetrachloride (CCl_4) on red blood cell numbers. Group 3, which received CCL4, exhibited reduced RBC levels in comparison to the control group. This outcome aligns with the findings of [11], which identified a decrease in hemoglobin levels, white blood cells, and red blood cells. The reduction is attributed to hemolysis of erythrocytes and circulatory system disturbances induced by the compound's elevated toxicity [12]. The impact of the treatment agent, an aqueous extract of Capparis spinosaon, on the RBC levels of Group 2 is illustrated in the same image, which was administered both the therapeutic aqueous extract and the deleterious toxin (CCL4). Compared to the other groups, the data indicates that this group's RBC levels have risen. The enhancement is attributed to the alkaloids, glycosides, saponins, and flavonoids in the caper extract, which bolster the immune system and reduce oxidative stress [11].

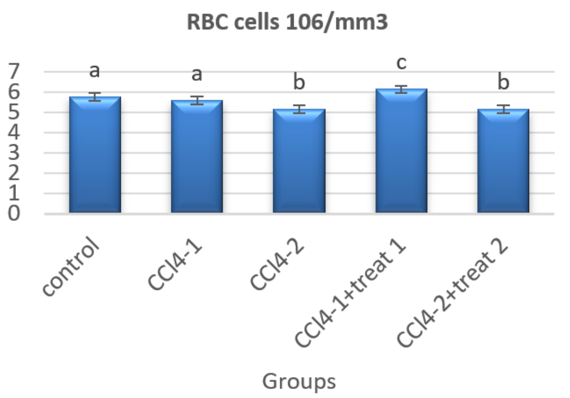

Figure 1: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on RBC levels.

Group 3, which received CCL4, exhibited decreased hemoglobin levels compared to the other groups (Fig. 2). This decline corresponds with data from reference [11], which observed a decrease in hemoglobin resulting from hemolysis induced by CCL4 poisoning. The same figure demonstrates that the therapeutic aqueous extract did not significantly influence hemoglobin levels, since Group 3 had a continued decline in hemoglobin post-treatment relative to the other groups (Fig. 2).

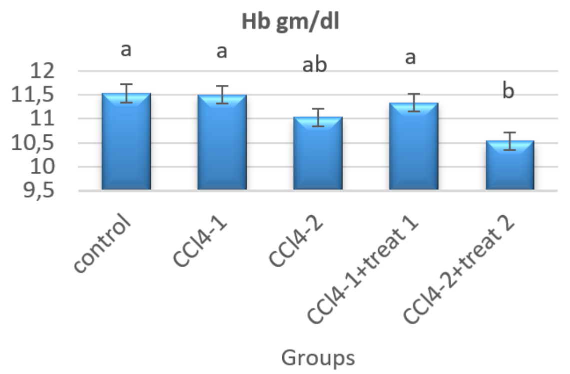

Figure 2: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on hemoglobin (hb) levels.

Figure 3 illustrates a reduction in white blood cell (WBC) levels in Groups 2 and 3, which received CCL4, in contrast to the results from reference [11], ascribed to hemolysis and circulatory disruptions resulting from CCL4 toxicity. The same figure demonstrates that the therapeutic aqueous extract elevated WBC levels in Group 2 relative to the other groups, attributable to its constituents of alkaloids, glycosides, saponins, and flavonoids, which mitigate oxidative stress and bolster the immune system [13]. To the control group. This reduction is steady.

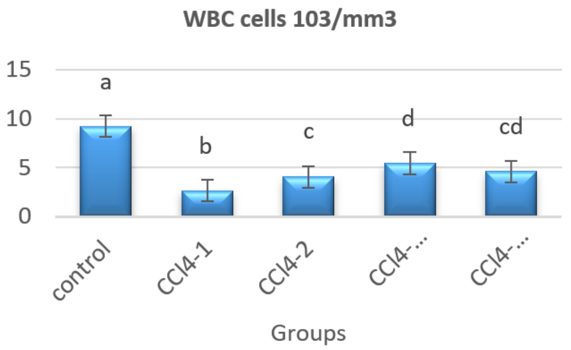

Figure 3: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on WBC levels.

Figure 4 illustrates a reduction in lymphocyte levels in Group 3 relative to the other groups. Group 2, which received only CCL4, exhibits elevated lymphocyte counts in comparison to the control group. This drop corresponds with study [11], which reported a decline in white blood cells, including lymphocytes, attributable to hemolysis and circulatory disruptions caused by CCL4 toxicity [14]. The same figure demonstrates that the therapeutic aqueous extract of the caper plant elevated lymphocyte counts in Group 3, which received both CCL4 and the caper extract, in comparison to Group 2. This is ascribed to the alkaloids, glycosides, saponins, and flavonoids in caper extract, which mitigate oxidative stress and bolster the immune system. Research indicates that the caper herb may enhance immune function, augmenting the body's capacity to combat infections and viruses while boosting cytokine activity, which is crucial for immune response and includes lymphocytes. [5]

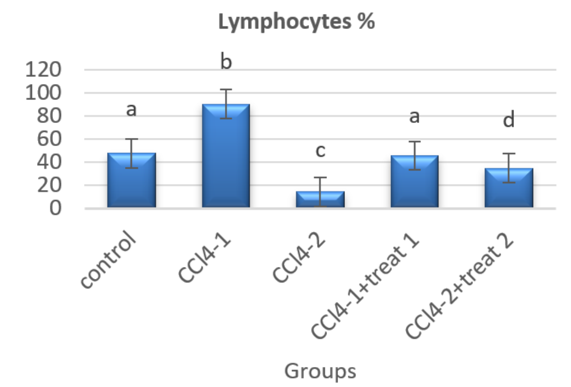

Figure 4: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on lymphocytes.

Figure 5 illustrates a notable reduction in platelet count in both Groups 2 and 3, who received CCL4 treatment, in comparison to the control group. This decrease is ascribed to hemolysis and circulatory disruptions induced by CCL4 poisoning [5].

The figure illustrates that the therapeutic aqueous extract of the caper plant elevated platelet levels in Group 2, which was administered both CCL4 and the extract. This increase indicates a restoration of normal platelet numbers attributable to the caper extract's alkaloids, glycosides, saponins, and flavonoids, which mitigate oxidative stress [9].

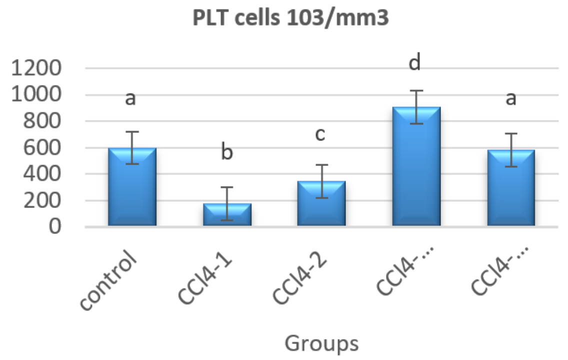

Figure 5: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on platelet count.

Figure 6 illustrates an elevation in ALT enzyme levels in Groups 2 and 3, who received CCL4, in contrast to the control group. This increase is attributable to the harmful effects of CCL4 on hepatic enzymes, resulting in alterations in lipid metabolism and tissue damage (2). The same figure demonstrates that the therapeutic aqueous extract of the caper plant lowered ALT levels in Groups 2 and 3, approaching normalcy. This reduction corresponds with study [8], which shown that caper extract reduced ALT activity in carbon tetrachloride-induced toxicity owing to its alkaloids, glycosides, saponins, and flavonoids that mitigate oxidative stress and bolster immunological function. [14].

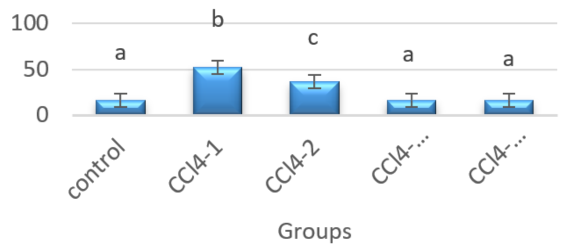

Figure 6: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on ALT enzyme levels.

Figure 7 illustrates an elevation in AST enzyme levels in Group 2, which received CCL4 prior to treatment, in comparison to the control group. This increase aligns with study [8], which documented a notable elevation in AST levels attributable to liver fibrosis produced by CCL4. The same figure demonstrates that, following treatment with the therapeutic aqueous extract of the caper plant, there was a significant increase in AST levels in Group 2.

The increase is ascribed to liver fibrosis in the rabbits, which did not adequately respond to the treatment [15].

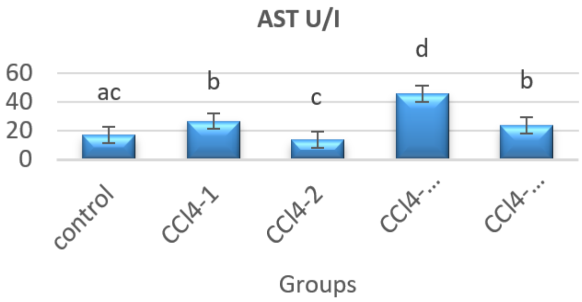

Figure 7: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on AST enzyme levels.

Figure 8 illustrates a reduction in ALP enzyme levels in both Groups 2 and 3, who received CCL4, in comparison to the control group. This reduction may be attributed to a shortage in phosphates, zinc, or vitamin C, or it may stem from diseases such as osteomalacia or hepatic and renal failure. Exposure to CCL4 may result in elevated liver enzyme levels due to increased oxidative stress and free radical damage [16], [17]. The decrease in ALP levels may be associated with hepatocyte apoptosis and heightened oxidative stress, resulting in enzyme leakage into the plasma [18]. The same figure demonstrates that following treatment with the therapeutic aqueous extract of the caper plant, ALP levels were normalized in both Group 2 and Group 3. This enhancement indicates that the caper extract supports liver and kidney function, elevates antioxidant levels, and diminishes fat levels owing to its flavonoid constituents. This conclusion is corroborated by study [15], which demonstrated that caper extract possesses antioxidant characteristics that aid in the regulation of enzyme levels.

Figure 9 illustrates a reduction in glucose levels in Groups 2 and 3, who received CCL4, in comparison to the control group. This conclusion contradicts study [19], which indicated an elevation in glucose levels in rats administered CCL4 relative to the control group. The noted reduction in glucose levels may arise from CCL4's suppression of glucose-6-phosphate dehydrogenase activity, maybe attributed to diminished microsomal enzyme activity due to the compound's toxicity [20]. The same figure demonstrates that therapy with the therapeutic aqueous extract of the caper plant enhanced glucose levels in mice subjected to the harmful chemical. Research indicates that caper extract is advantageous for diabetic individuals by decreasing blood glucose levels, elevating good lipids, and diminishing triglyceride levels. This enhancement corresponds with study [21], which discovered that caper extract positively influences glucose control and lipid profiles in diabetes circumstances.

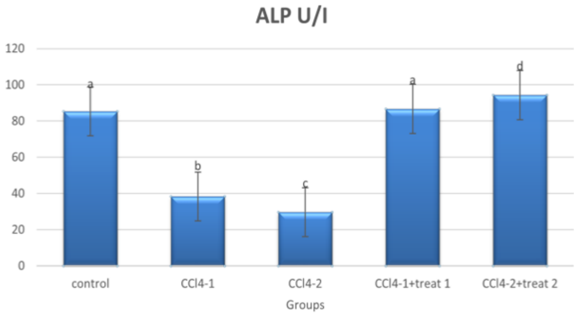

Figure 8: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on ALP enzyme levels.

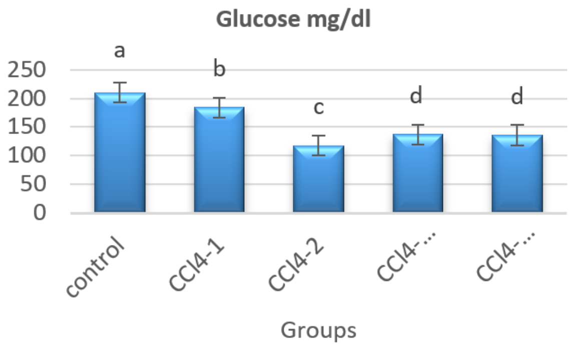

Figure 9: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on glucose levels.

Figure 10 demonstrates a reduction in cholesterol levels in both Groups 2 and 3, who received CCL4 without therapy, in comparison to the control group. This result aligns with study [22], which showed that increasing doses of CCL4 resulted in heightened toxicity, including liver enlargement, hepatic steatosis, increased cholesterol levels, renal toxicity, and diminished protein levels. The same figure demonstrates that the therapeutic aqueous extract of the caper plant efficiently corrected cholesterol levels in Groups 2 and 3, which were administered both CCL4 and the caper extract. This enhancement corresponds with study [23], which shown that caper extract diminishes detrimental cholesterol levels in the bloodstream, specifically low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL), linked to an elevated risk of thrombosis.

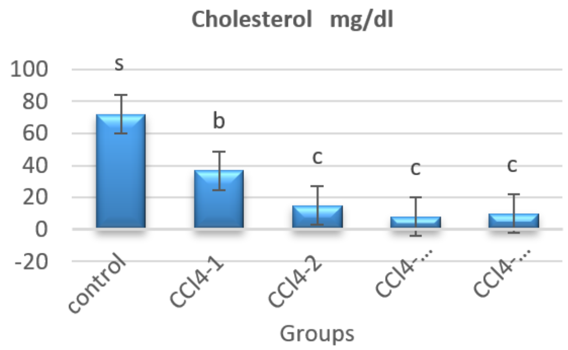

Figure 10: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on cholesterol levels.

Figure 11 illustrates an elevation in triglyceride (TG) levels in Group 3, which received CCL4, in contrast to the control group. This rise is ascribed to CCL4-induced hepatic injury, including necrosis and toxic repercussions, resulting in elevated TG levels. This conclusion aligns with studies [19,24], which noted a substantial increase in triglyceride levels in CCL4-treated groups, contributing to non-alcoholic fatty liver disease. The elevation in triglycerides results from the disruption of lipid metabolism and heightened blood triglyceride concentrations impacting tissue lipid levels. The same figure demonstrates that the therapeutic aqueous extract of the caper plant markedly diminished TG levels in Group 2, which received both CCL4 and the caper extract, in comparison to pre-treatment levels. This indicates that the caper extract aids in alleviating the lipid abnormalities induced by CCL4 exposure.

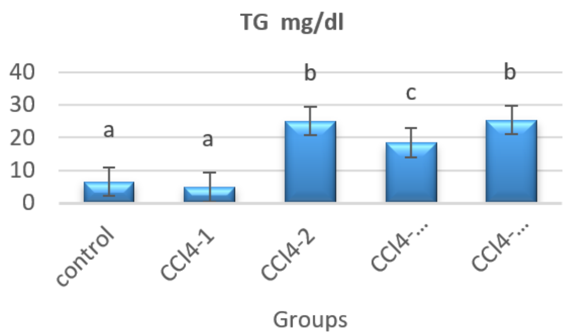

Figure 11: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on triglyceride (TG) levels.

Figure 12 illustrates a reduction in total protein levels in Groups 1 and 2, which received CCL4, in comparison to the control group. This decrease is ascribed to diminished protein levels in the liver resulting from CCL4 exposure, causing hepatic damage. This disorder leads to diminished total protein levels in the blood, including reductions in amino acids such as tyrosine and glutamine, and may also result in lower protein levels due to inflammation in the small intestine impacting absorption. This conclusion aligns with study [23], which documented a comparable reduction in total protein levels attributable to CCL4 toxicity. The same figure demonstrates that the therapeutic aqueous extract of the caper plant enhanced total protein levels in the treated groups. This enhancement is ascribed to the antioxidant characteristics of caper extract, which aid in lowering lipid levels and augmenting overall antioxidant levels, hence improving protein status [25]. This impact is corroborated by study [26], which emphasized the caper plant's contribution to enhancing antioxidant defenses and optimizing metabolic activities.

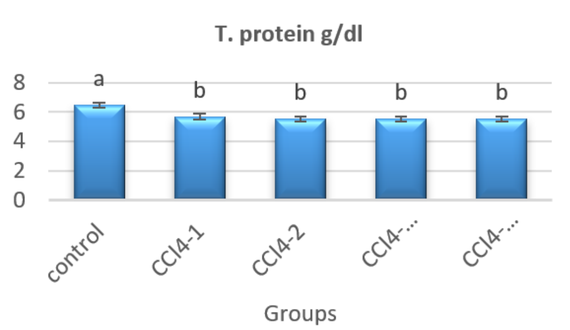

Figure 12: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on total protein levels.

Figure 13 illustrates a reduction in urea levels in Groups 2 and 3, who received CCL4, in comparison to the control group. This data contradicts study [22], which indicated that CCL4 exposure generally results in nephron destruction, renal failure, and an ensuing elevation in urea levels due to kidney dysfunction. The noted reduction in urea levels may be ascribed to initial renal impairment, which influences urea synthesis and elimination. The same figure demonstrates that the inclusion of the therapeutic aqueous extract of the caper plant enhanced urea levels in Group 3 relative to the other groups. This enhancement indicates that the caper extract aids in maintaining renal function and elevating antioxidant levels, which may mitigate some of the damage inflicted by CCL4. The caper plant's capacity to sustain liver and kidney functions, along with its antioxidant characteristics, as indicated in study [15], aids in the normalization of urea levels.

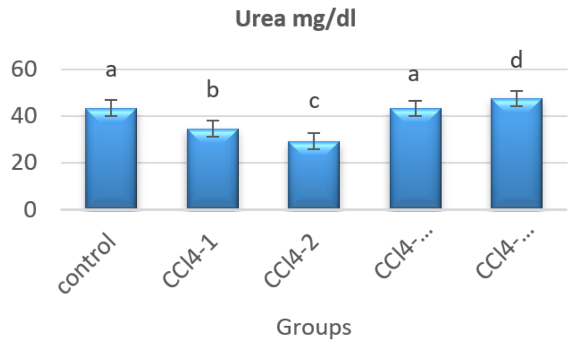

Figure 13: Effect of carbon tetrachloride (CCL4) and the therapeutic agent on urea levels.

4 CONCLUSIONS

The present study concludes that the administration of CCL4 at doses of 0.2 and 0.3 mL/kg body weight produced substantial effects on red blood cells, white blood cells, hemoglobin, lymphocytes, and platelets. Additionally, it caused changes in liver enzymes, cholesterol, triglycerides, urea, and total protein levels. Treatment with an aqueous extract of the Capparis spinosa demonstrated a partial ability to mitigate the toxic effects of CCl_4. Some hematological and biochemical indicators improved, particularly red and white blood cell counts, lymphocyte counts, cholesterol and glucose levels, and certain liver enzymes. This reflects the protective role of the plant's active compounds, such as flavonoids, alkaloids, saponins, and phenolic compounds, known for their antioxidant and immunomodulatory properties. However, some adverse effects persisted after treatment, especially regarding urea, hemoglobin, triglyceride, and certain liver enzyme levels. This suggests that the plant extract's protective effect was only partial and did not fully restore all indicators to normal levels. This may be due to the severity of the oxidative damage caused by CCl_4 or to the limited duration or dosage of the extract used.

Therefore, it can be concluded that the aqueous extract of Capparis spinosa possesses a partial protective effect against CCl_4-induced toxicity and is a promising candidate for use as a natural therapeutic supplement to support liver and blood function. However, further, larger-scale future studies involving varying dosages and longer treatment periods, along with a more in-depth investigation of the molecular mechanisms of action of the plant extract, are required.

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