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Hepatoprotective efficacy of *Lagenaria siceraria* seeds oil against experimentally carbon tetrachloride-induced toxicity

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ABSTRACT

Background: The liver is crucial for maintaining normal metabolism in the body. Various substances, such as toxic chemicals, drugs, and alcohol, can damage hepatocyte cells, leading to metabolic imbalances.

Aim: The experiment aimed to determine the efficacy of *Lagenaria siceraria* seed oil (LSS) as a hepatoprotective agent against acute hepatotoxicity triggered by carbon tetrachloride (CCl_4).

Methods: A total of 20 rats were randomly separated into four groups. The control group: rats received 2 ml of distilled water orally, followed by 1.25 ml of olive oil intraperitoneally (i.p.) after 30 minutes. CCL_4 group: rats were given a single intraperitoneal dose of 1.25 ml/kg b.w. of CCl_4 in a 1:1 mixture with olive oil. Silymarin group: received 100 mg of silymarin per kg of b.w. diluted in 2 ml of distilled water orally, followed by CCl_4 treatment after 30 minutes. LSS oil group: received LSS oil at 3g/kg b.w. orally, followed by CCl_4 treatment after 30 minutes. Blood samples were collected to assess liver enzymes (AST, ALT, and ALP), proteins and bilirubin fractions, and redox status (catalase, reduced glutathione (GSH), and malondialdehyde (MDA)) were assessed in hepatic tissues. Changes in liver histopathological examination were also evaluated.

Results: In CCl4-treated rats, there was a significant increase in serum liver marker enzyme activity (ALP, AST, and ALT) along with a significant elevation ($p < 0.05$) in total bilirubin, indirect bilirubin, and direct bilirubin compared to the control rats. However, all these parameters decreased in the CCl_4+ Silymarin and CCl_4+LSS groups compared to CCl_4-treated rats. There was a significant decline in total protein level and serum albumin in all experimental groups compared to the control, while globulin levels significantly increased in all experimental groups. There was a significant ($p < 0.05$) reduction in the level of GSH and catalase, with an increase in MDA level in CCl_4 rats compared to other rats. Histopathological investigation of the LSS-treated group showed a hepatoprotective effect against CCl_4.

Conclusion: The study revealed that LSS oil has antioxidant activity against CCl_4-induced toxicity.

Keywords: Antioxidant activity, *Lagenaria siceraria* seed oil, Carbon tetrachloride, Hepatotoxicity.

Introduction

The liver is crucial for maintaining normal metabolism in the body. Various substances, such as toxic chemicals, drugs, and alcohol, can damage hepatocyte cells, leading to metabolic imbalances (Zheng *et al*., 2022). Exposure to toxic chemicals can weaken the antioxidant system and lead to an overproduction of oxidative stress (OS). OS plays a role in promoting various health issues, including liver damage (Arroyave-Ospina *et al*., 2021). Additionally, the decline of antioxidant defenses in the cellular system may lead to liver dysfunction (Sadasivam *et al*., 2022). Carbon tetrachloride (CCl_4) is a potent environmental hepatotoxin commonly applied to prompt liver impairment in experimental animal models to assess the effectiveness of hepatoprotective drugs (Zahoor *et al*., 2021; Wang *et al*., 2023).

OS-triggered by CCl_4 can damage unsaturated fatty acids in the membrane, leading to peroxidation reactions that affect membrane permeability and fluidity, causing OS. Moreover, CCl_4 is a toxic compound that causes chemical hepatitis and liver damage in animals, commonly used in experiments to study liver damage and check the effectiveness of potential treatments. A single exposure to CCl_4 can

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cause severe hepatic necrosis (Brautbar and Williams II, 2002; Manibusan *et al*., 2007). CCl_4 induces cell destruction by forming covalent bonds with cellular components or by increasing lipid peroxidation, which breaks down unsaturated fatty acids and damages unsaturated phospholipids, leading to intracellular plasma membrane damage (Cheeseman *et al*., 1985). Liver toxicity from CCl_4 is exacerbated by low tissue oxygen levels, which promote the formation of harmful radicals and metabolite binding. Although traditional liver medications are effective, their long-term side effects are worrisome (De Groot *et al*., 1988; Yasusuke and Yumi, 1990). Therefore, investigating natural substances as potential alternatives to synthetic hepatic protectors is crucial. Phytochemicals contain many active compounds used for centuries to treat various diseases (Abd El-Hack *et al*., 2016, 2019, 2020a,b, 2023). *Lagenaria siceraria* (Yaqtin, LSS) is a plant from the Cucurbitaceae family, commonly used in traditional medicine. The fruit of LSS is known for its antipyretic and diuretic properties. A water extract of LSS leaves mixed with sugar treats jaundice (Panchal *et al*., 2013).

Lagenaria siceraria is a palatable fruit and a fitting source of vitamin B-complex, C, β-carotene, and pectin. It also contains superior choline levels, a lipotropic compound (Singh *et al*., 2010). The active compounds in LSS, such as saponins, phenolic, and alkaloids, have exhibited antioxidant, anti-inflammatory, and hepatoprotective effects (Rahman, 2003; Singh *et al*., 2010; Zahoor *et al*., 2021). The seeds of LSS fruits are used as a remedy for cough, fever, and earache. They are also considered a brain tonic and have anti-inflammatory properties (Saeed *et al*., 2022). These fruits contain saponins, previously studied for their hepatoprotective properties (Panchal *et al*., 2013). Natural phytochemicals have garnered significant interest in recent years due to their various beneficial bioactivities (Zahoor *et al*., 2021). Based on the antioxidant action of LLS reported in previous studies, we hypothesized that LLS could protect against liver damage induced by CCl_4. In this experiment, we aimed to investigate the hepatoprotective properties of LSS on CCl_4-induced hepatic tissue damage and explore the underlying mechanisms of its hepatoprotective action by examining blood health, OS, and antioxidant efficacy, using Silymarin as a standard hepatoprotective drug.

Materials and Methods

Chemicals and material sources

Lagenaria siceraria (LSS) pale yellow and clear oil was obtained from Zagazig University, College of Agriculture. CCl_4 is a colorless, clear, volatile, and constant chlorinated hydrocarbon (Alexandria Chemical Company, Egypt). Doxorubicin, with the trade name ADRIAMYCIN, is a 5 ml vial with a concentration of 2 mg/ml and is the registered trademark of Pharmacia and Upjohn Company. The commercial kits for detecting liver enzymes, total proteins, albumin, bilirubin, direct bilirubin, glutathione (GSH), catalase, and MDA were obtained from bio-diagnostics Company (Cairo, Egypt). All chemicals used in the research were of analytical grade quality.

Animals and experimental procedures

Twenty healthy Wistar rats weighing 190 ± 10 g were acquired from the Veterinary Medicine Faculty Farm, Zagazig University, and registered for this experiment. They were accommodated in cages with controlled humidity (50%–70%), temperature (25°C ± 2°C), and a 12-hour light/dark cycle, free to water and feed *ad libitum*. The rats were monitored for abnormal behavior and clinical signs throughout the experiments. After a one-week acclimatization period, the rats were randomly divided into four groups of five rats each, as follows:

1. The first group served as the control group and received 2 ml of distilled water orally, followed by 1.25 ml of olive oil intraperitoneally (i.p.) after 30 minutes.
2. The second group received a single intraperitoneal dose of CCL_4 1.25 ml/kg b.wt. in olive oil (1:1) according to the method of Khan and Alzohairy (2011).
3. The third group received 100 mg of Silymarin/kg b.wt. diluted in 2 ml distilled water orally, following the method of El-Maddawy and Gad (2012), and then were treated with CCL_4 as in the second group at 30 minutes, 12 hours, and 24 hours.
4. The fourth group received LSS oil at 3g/kg b.wt. orally as recommended by Kumar *et al*. (2012) and Shendge and Belemkar (2021), followed by CCL_4 treatment in the second group at 30 minutes, 12 hours, and 24 hours.

After 24 hours of CCl_4 injection, all rats were sacrificed under anesthesia, and blood samples were collected from the retro-orbital venous plexus for assessing blood biochemistry. The hepatic tissues were then excised and kept in 10% of paraformaldehyde (PFA) for further histology screening.

Blood and tissue sampling

After thirty-six hours of the experiment, the animals were anesthetized with diethyl ether. Blood was collected from the retro-orbital venous plexus vein into sterilized test tubes for serum separation. The samples were centrifuged at 3,000 rpm for 15 minutes and stored at −20°C until biochemical examination. The rats were euthanized by cervical decapitation. The hepatic samples were excised and washed with physiological saline (NaCl 0.9%) and kept in 4% PFA for histopathological examination. Another part of the hepatic tissues was kept for further catalase, MDA, and GSH assays.

Serum biochemical analysis

The total proteins and albumin were assessed using a colorimetric method with commercial kits provided by Bio-diagnostics Company (Cairo, Egypt). The globulins were calculated based on the total proteins and albumin differences. The A/G ratio was determined by dividing albumin by globulins.

The serum ALT and AST corresponded to the assay described by Tietz (1986), and ALP was determined according to Kind and King (1954). The total bilirubin, direct bilirubin, and indirect bilirubin levels were measured following the guidelines by Abd El-Hack *et al*. (2019) and Ismail *et al*. (2019).

GSH, MDA, and catalase determination in hepatic tissues

The liver specimens were washed several times with PBS (0.1M, pH 7.4). Subsequently, 10% homogenates were prepared from hepatic tissues employing a disposable homogenizer tissue (Biomasher; Nippi, Inc., Tokyo, Japan) in cold potassium phosphate buffer (50 mM, pH 7.5) as described in the paper by Zhao *et al*. (2018). The homogenates were then centrifuged at 2000×g at 4°C for 15 minutes. The supernatants were collected for the estimation of malondialdehyde (MDA, a lipid peroxidation indicator) (Guarnieri *et al*., 1980; Renaudin, 2001) and antioxidant biomarkers such as catalase (Aebi, 1984) and GSH activities (Konrad *et al*., 1972), following the producer's guidelines (Bio-diagnostics Company Cairo, Egypt).

Histopathological analysis

The hepatic samples preserved in formalin were subjected to fixation and dehydration processes. The fixation involved immersing the tissue in 10% buffered formalin for three days with media change every 24 hours, followed by a 30-minute rinse in distilled water. Dehydration was carried out by sequential immersion in alcohol solutions (70%, 80%, 90%, and 100%) for 120, 90, 90, and 60 minutes, respectively. Subsequently, the tissues were cleared in xylene through a series of steps, including immersion in a 50% alcohol and 50% xylene mixture for an hour, followed by pure xylene for one and a half hours. The samples were then impregnated with molten paraffin wax, embedded, and sectioned. The paraffin sections (4–5 µm) were stained with hematoxylin and eosin (Suvarna and Layton, 2013) to evaluate fibrosis, steatosis, necrosis, or degenerative pathological lesions in the hepatic tissues.

Statistical analysis

Numbers are depicted as mean ± SEM and were analyzed using one-way analysis of variance, followed by Tukey's post-hoc test. Results were considered statistically significant at $p < 0.05$.

Ethical approval

The Authors' Institution Ethics Committee (IACUC) approved the study for animal studies at the Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt. Care was taken to minimize the number of animals used. The animal experiments followed the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by Zagazig University authorities.

Results

Serum biochemical restored by LLS administration

Compared to the control rats, the total protein level showed a significant reduction ($p < 0.05$) in animals injected with CCl_4 alone (14%) and CCl_4+ Silymarin (2%). At the same time, there was no substantial change between the standard and CCl_4+LSS oil groups (Fig. 1). The level of serum albumin significantly decreased (*p*

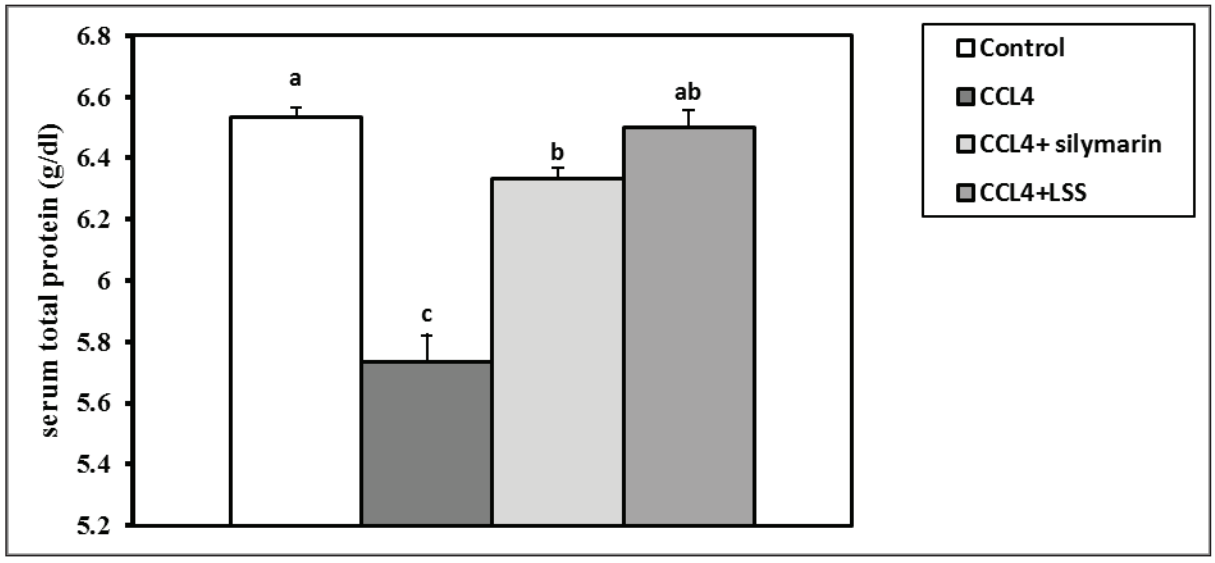

Fig. 1. Effect of *Lagenaria siceraria* seed oil (LSS), CCL_4 and Silymarin on serum total protein concentration in male rat. [a,b,c]: columns labeled with different letters display significant variations ($p<0.05$).

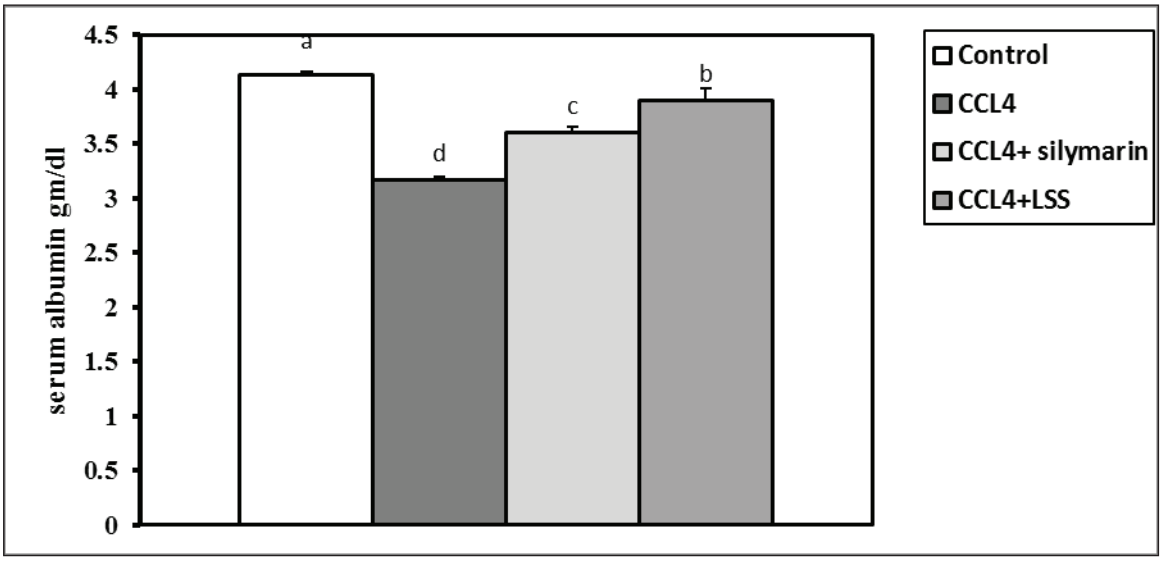

Fig. 2. Effect of LSS, CCL_4 and Silymarin on serum albumin gm/dl in male rat. [a,b,c,d] : columns labeled with different letters display significant variations (p<0.05).

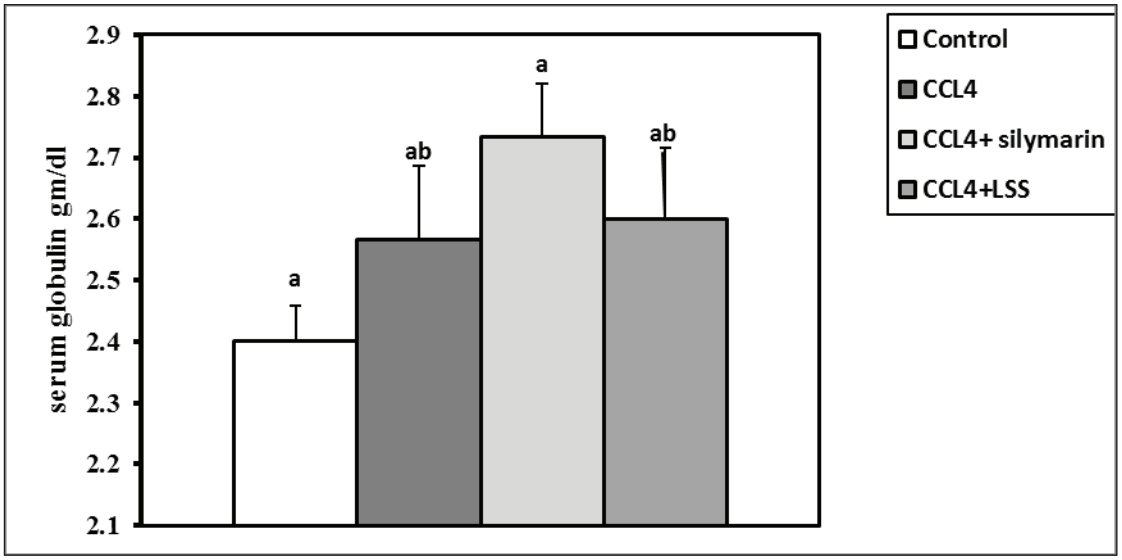

Fig. 3. Effect of LSS, CCL_4 and Silymarin on serum globulin gm/dl in male rat. [a,b]: columns labeled with different letters display significant variations (p<0.05).

< 0.05) in the CCl_4, CCl4+ Silymarin, and CC_{l4}+LSS groups (23.5%, 12.8%, and 5.5%), respectively (Fig. 2). Meanwhile, the globulin level notably boosted ($p < 0.05$) in the experimental groups (6.6%, 13.87%, and 8.33%), respectively (Fig. 3).

The albumin/globulin ratio was substantially heightened in the CCl_4 group, followed by the CCl_4+Silymarin-treated animals. In contrast, there was no substantial diversity between the control and CCl4+LSS oil-treated rats (Fig. 4). The CCl4 group showed a highly significant increase ($p < 0.05$) in liver marker enzyme activity (ALP, AST, and ALT) compared to the control. At the same time, there was a moderate increase in CCl_4+ Silymarin and CCl_4+LSS oil-treated rats compared to the control (Figs. 5–7). Results in (Figs. 8–10) showed the effect of CCl_4 administration with or without Silymarin or LSS on the serum level of indirect and direct bilirubin and total bilirubin, which confirmed a significant increase ($p < 0.05$) in total bilirubin, indirect bilirubin, and direct bilirubin in the CCl_4 group compared to the control and other treated groups. There were no substantial changes between CCl_4+ Silymarin and CCl_4+LSS oil-treated rats in indirect and direct bilirubin. Additionally, the results

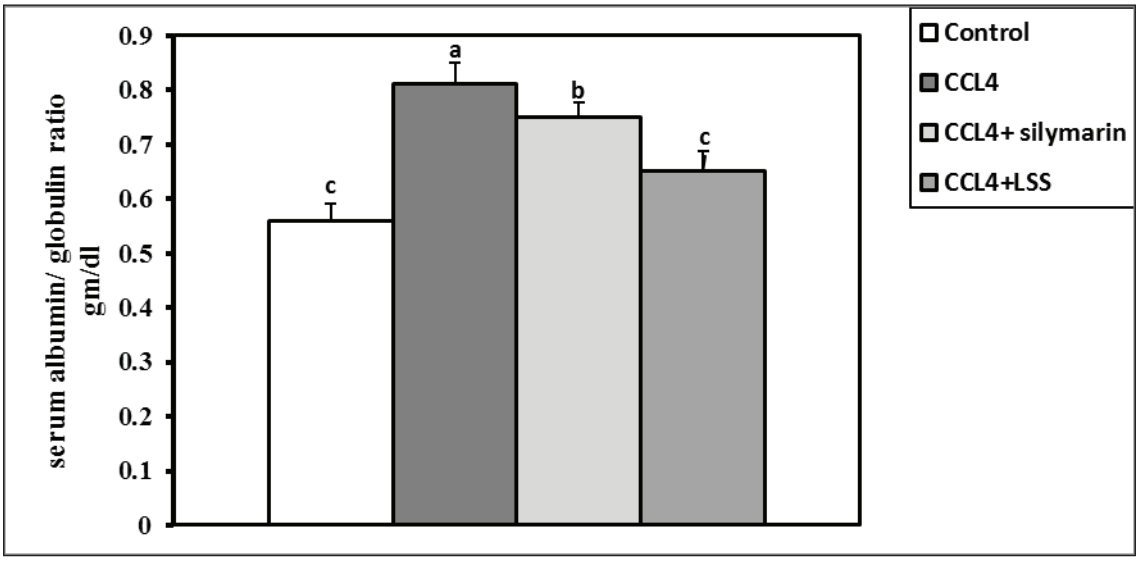

Fig. 4. Effect of LSS, CCL_4 and Silymarin on serum albumin/globulin ratio gm/dl in male rat. [a,b,c]: columns labeled with different letters display significant variations (p<0.05).

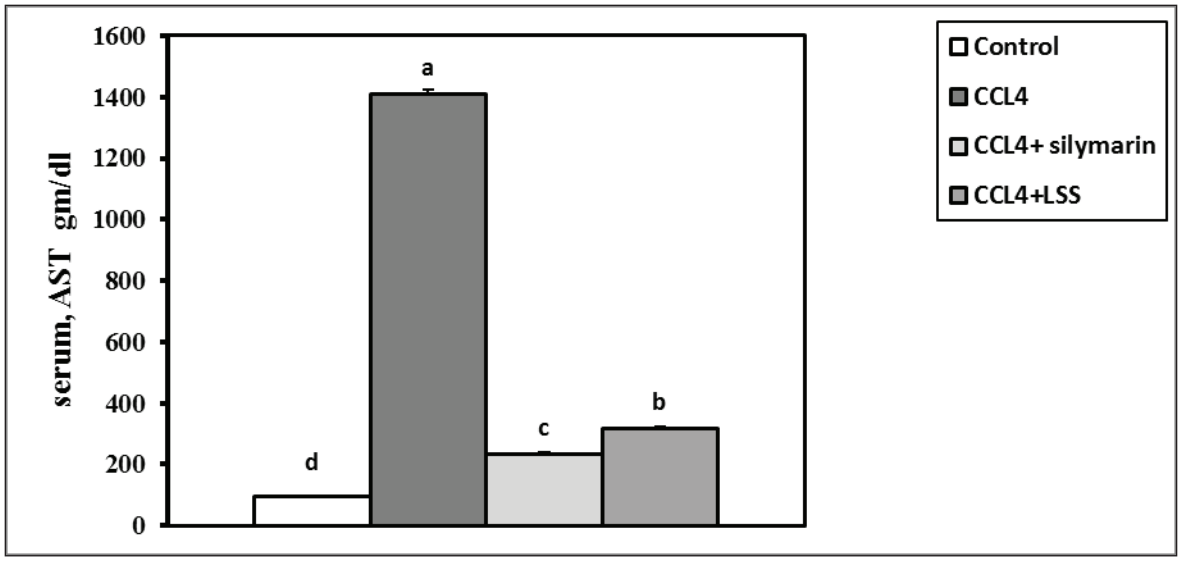

Fig. 5. Effect of LSS,CCL_4 and Silymarin on Serum AST gm/dl in male rat. [a,b,c,d] : columns labeled with different letters display significant variations (p<0.05).

showed no significant changes between the CCl_4+LSS and control group in total bilirubin.

LSS protected CCl4-induced liver damage via boosting antioxidant markers and reducing MDA

MDA levels increased significantly by 137.7% (Fig. 11). The results demonstrate the effects of treatments on antioxidant activities and the positive influence of LSS. It is clear that CCL_4 injection significantly decreased the values of catalase by 36% (Fig. 12). GSH by 68% (Fig. 13). There was no significant difference between the CCl4+Silymarin and CCL4+LSS groups in terms of catalase and GSH levels. Still, both groups demonstrated a substantial improvement related to CCL_4-treated rats.

Histopathological analysis after administration with LSS

The control group examined liver sections that showed normal hepatic parenchyma with preserved lobular arrangement, hepatic cords, sinusoids, and vascular tree (Fig. 14A and B). Sections from the livers of CCL_4-treated rats (Fig. 14C and D) exhibited a large number of hepatocytes (70%–75%), particularly in a periportal

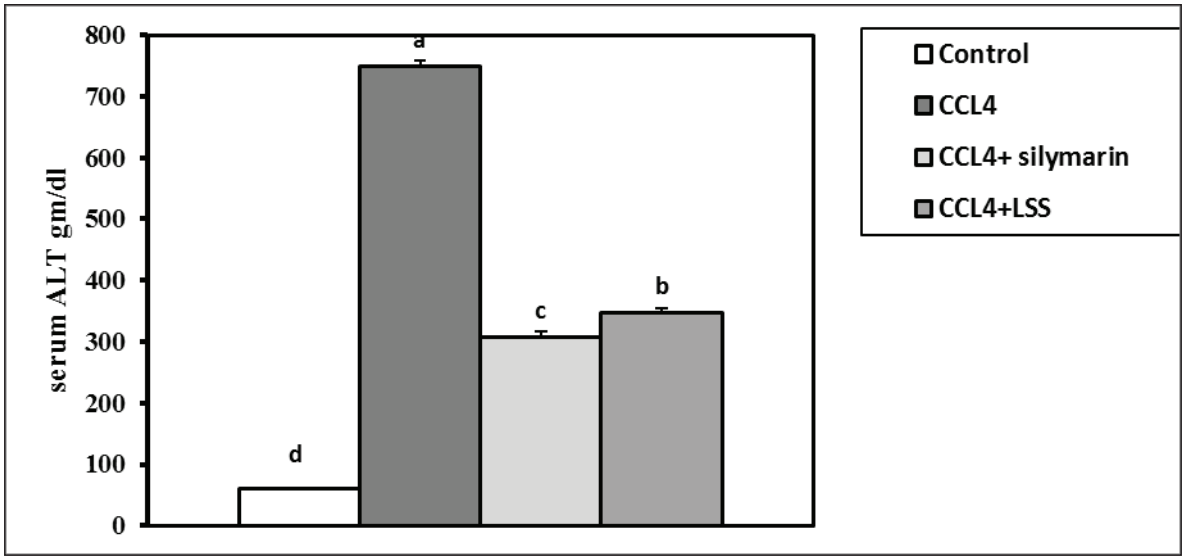

Fig. 6. Effect of LSS, CCL_4 and Silymarin on Serum ALT gm/dl in male rat. [a,b,c,d] : columns labeled with different letters display significant variations (p<0.05).

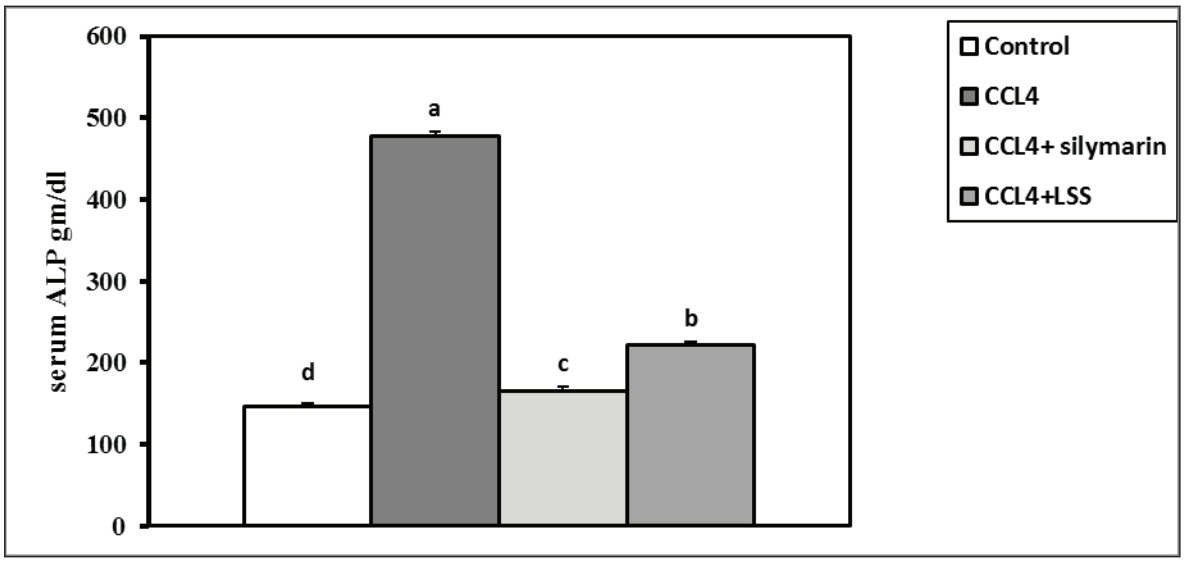

Fig. 7. Effect of LSS, CCL_4 and Silymarin on Serum ALP gm/dl in male rat. [a,b,c,d] : columns labeled with different letters display significant variations (p<0.05).

arrangement with micro and macrosteatosis, along with ballooning changes in a variable number of cells with centrally or eccentrically situated pyknotic nuclei. The portal triads displayed moderately congested blood vessels, moderate infiltration of lymphocytes and macrophages, and mild biliary hyperplasia. Focal necrotic and apoptotic changes in some hepatocytes were also observed (Fig. 14C and D).

On the other hand, sections from CCL_4 + Silymarin-treated rats (Fig. 14E and F) revealed a large to moderate number of hepatocytes (50%–60%) with micro and macrosteatosis and the presence of a few cells with ballooning changes. The portal triads exhibited moderate to severe congestion of portal blood vessels and round cell infiltration. The Kupffer cells appeared hypertrophied, and a few apoptotic hepatocytes were also seen (Fig. 14E and F). Examined liver sections from CCL_4 + LSS-treated rats (Fig. 14G and H) showed mild to moderate hydropic degeneration and fatty changes in hepatocytes (40%–45%), mostly in a centrilobular, peripherolobular, and periportal arrangement. The remaining hepatocytes displayed regenerative attempts with enlargement of the nuclei,

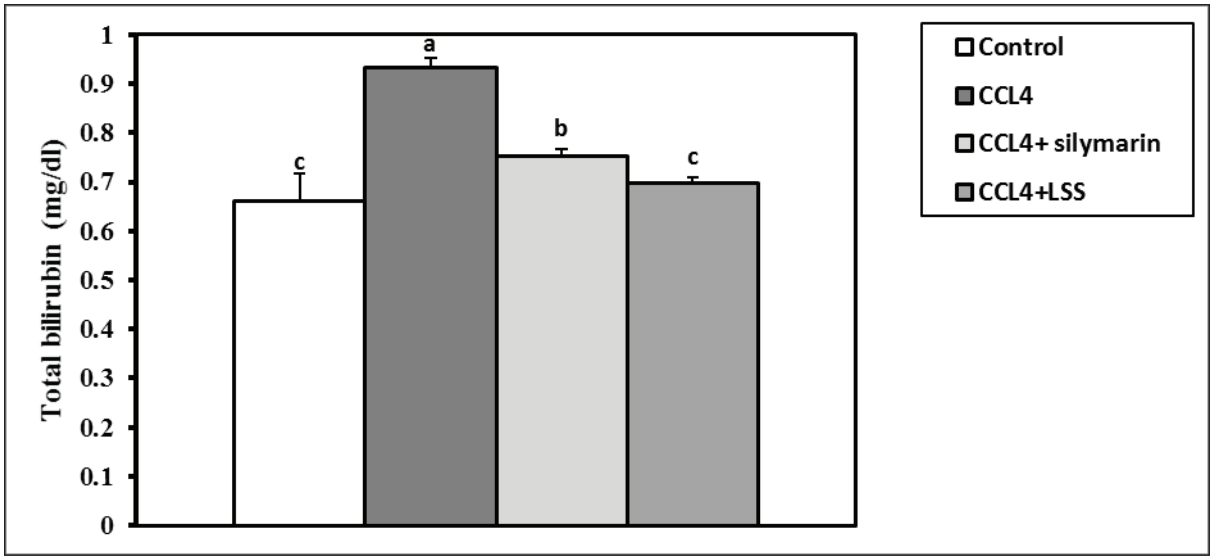

Fig. 8. Effect of LSS, CCL_4 and Silymarin on Serum total bilirubin gm/dl in male rat. [a,b,c]: columns labeled with different letters display significant variations (p<0.05).

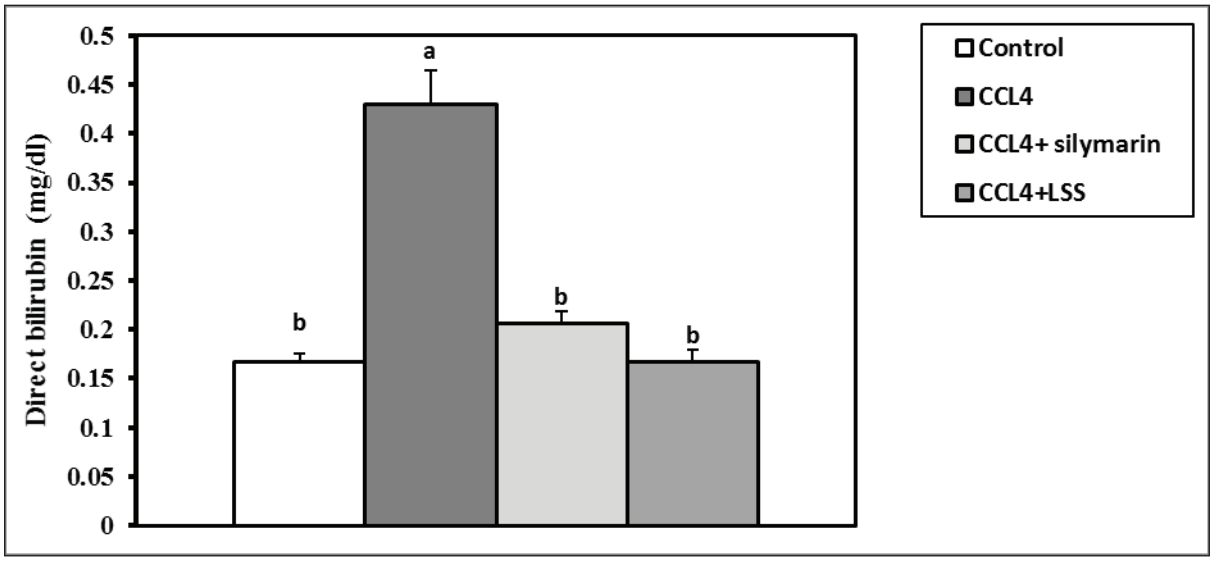

Fig. 9. Effect of LSS, CCL4 and Silymarin on serum direct bilirubin gm/dl in male rat. [a,b]: columns labeled with different letters display significant variations (p<0.05).

which appeared hyperchromatic or double-nucleated (Fig. 14G and H).

Discussion

The global burden of liver disease is significant, with over one million deaths annually. There is a need to discover effective off-the-shelf hepatoprotective agents. The current study shows that pre-treatment with LSS effectively reduces CCl_4-induced liver damage in a rat model. The LSS can effectively restore liver enzymes and redox imbalance induced by CCl_4. This study highlights the potential of LSS as a natural remedy for liver damage caused by toxic substances.

Lagenaria siceraria has been used in ointments to treat various diseases worldwide. The plant has antioxidant, free radical scavenging, cardioprotective, and hepatoprotective properties (Kumar *et al*., 2012; Zahoor *et al*., 2021). LSS is conventionally employed in liver syndromes and diseases induced by different free radicals (Saha *et al*., 2011b). Hepatocellular damage increases serum enzyme indices circulated from the hepatocytes into the blood (Sreelatha *et al*., 2009). CCl_4

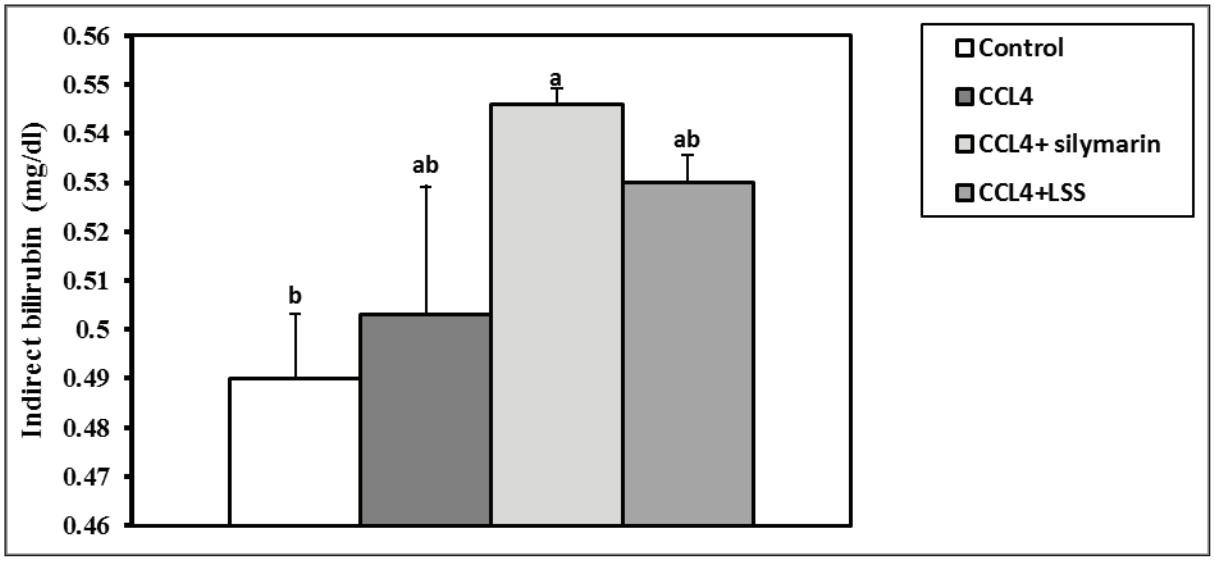

Fig. 10. Effect of LSS, CCL4 and silymarin on serum indirect bilirubin gm/dl in male rat. [a,b,c]: columns labeled with different letters display significant variations (p<0.05).

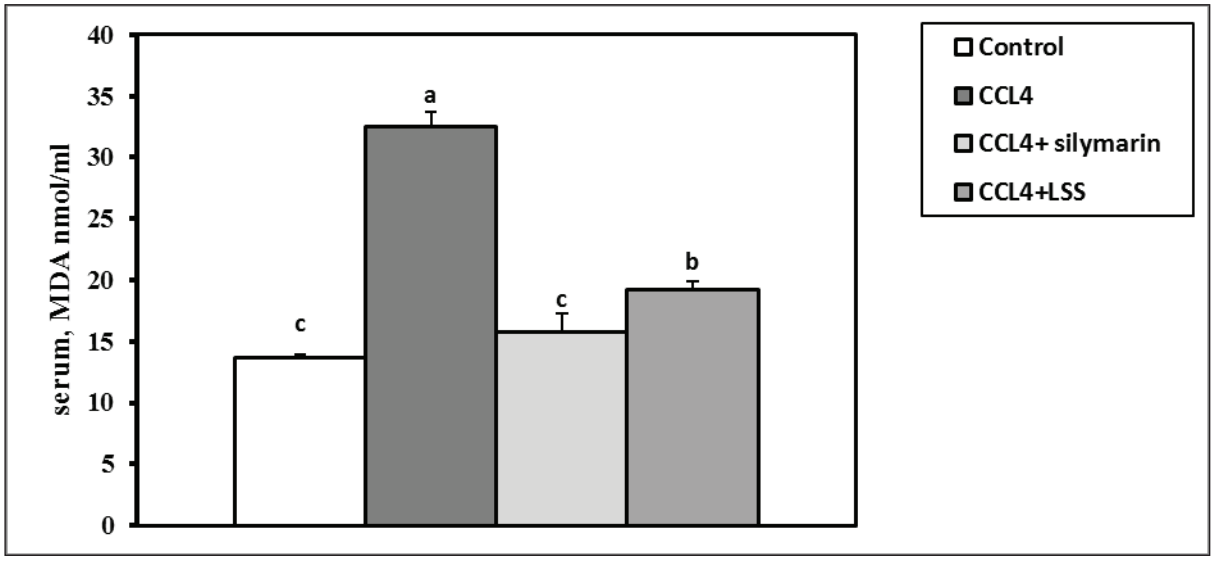

Fig. 11. Effect of LSS, CCL_4 and Silymarin on serum MDA nmol/ml in male rat. [a,b,c]: columns labeled with different letters display significant variations (p<0.05).

induces hepatotoxicity by forming a covalent bond with membrane proteins and causing lipid peroxidation (Kanter *et al*., 2005; Wang *et al*., 2023).

Liver tissue contains enzymes used as indicators to verify biological liver injury. The enzymes AST, ALT, and ALP are essential for evaluating liver injury (Wang *et al*., 2023). The hepatoprotective impact of *Lagenaria siceraria* extracts was assessed by examining liver function biochemical parameters (Saha *et al*., 2011a). CCL_4 has been shown to cause significant hepatic impairment, as indicated by a marked increase in serum ALP, AST, and ALT levels, which are indicators of cellular leakage and deficiency of functional reliability of hepatic cells (Althnaian *et al*., 2013). The existing report demonstrates the effect of CCL_4 on liver-specific enzymes, showing a significant elevation in serum liver enzyme activities in CCL_4-treated animals. Rats treated with CCL4, LSS, or Silymarin demonstrated a significant decrease in serum enzyme activities AST, ALT, and ALP. This research is consistent with earlier research by (Lakshmi *et al*., 2011), where the oral administration of the ethanolic extract of *Lagenaria siceraria* fruit (LSS) to various groups of rats decreased

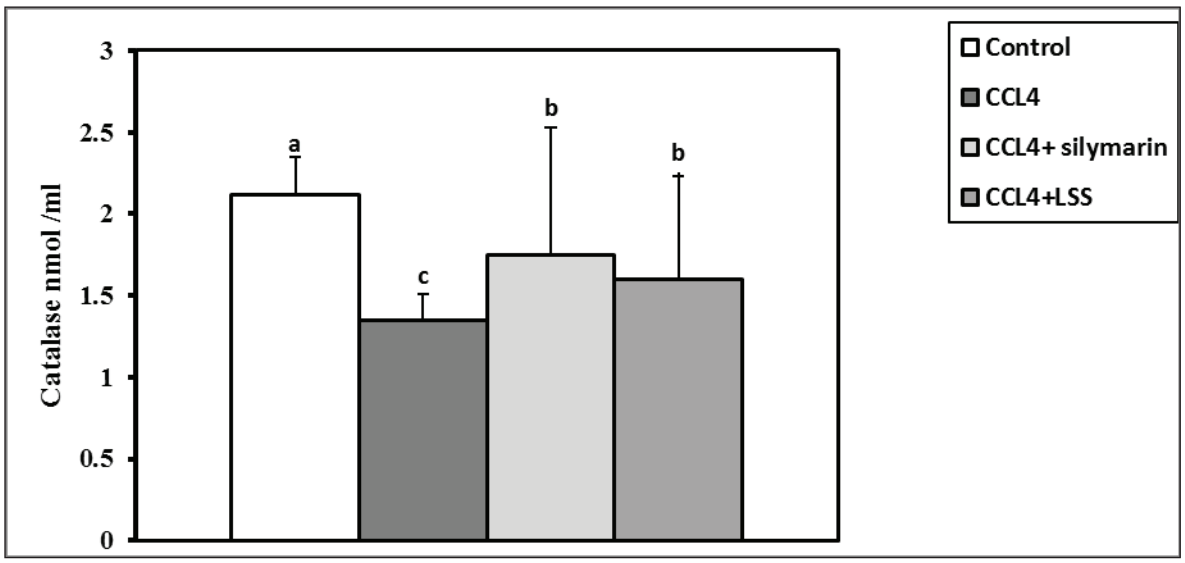

Fig. 12. Effect of LSS, CCL4 and silymarin on serum catalase nmol/ml in male rat. [a,b,c] : columns labeled with different letters display significant variations (p<0.05).

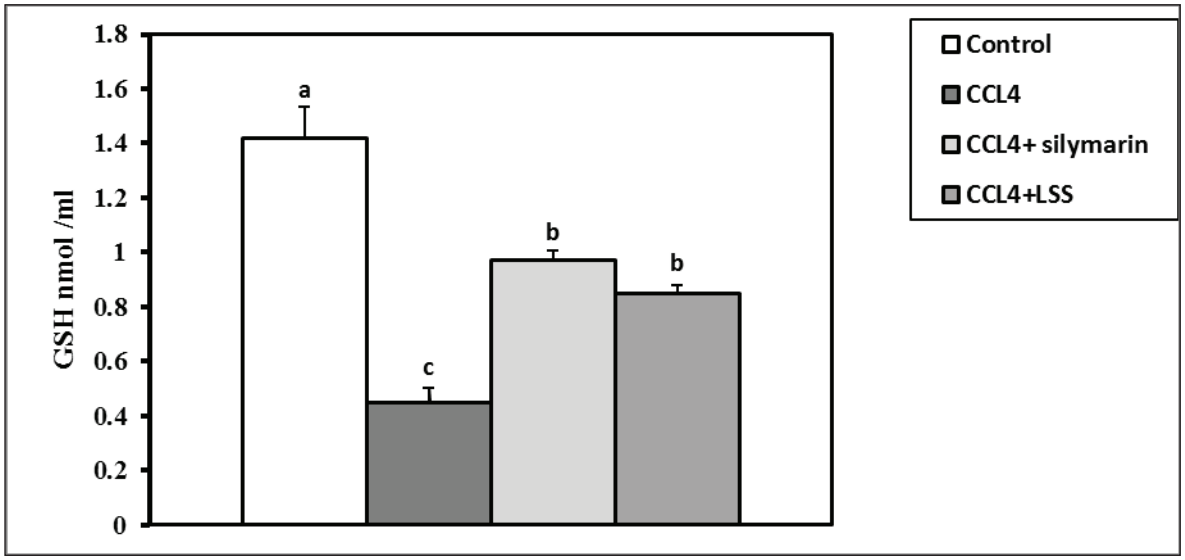

Fig. 13. Effect of LSS, CCL4 and Silymarin on Serum GSH nmol/ml in male rat. [a,b,c]: columns labeled with different letters display significant variations (p<0.05).

levels of serum ALT, AST, and ALP (Deshpande *et al*., 2008).

Our results were consistent with (Shirwaikar and Sreenivasan, 1996), who reported that the hepatoprotective action of *Lagenaria siceraria* extract was evaluated by studying its impact on hepatotoxicity induced by the administration of CCl_4, with Sylimarin used as a standard. Another biomarker for hepatotoxicity is the levels of total proteins and albumin, primarily produced by the liver. Hypoalbuminemia is commonly observed in advanced chronic liver diseases. In a recent study, acute administration of CCl_4 resulted in significant hepatic damage, as evidenced by the decrease in serum albumin, total proteins, globulin levels, and A/G ratio. This decline was attributed to the disassociation of polyribosomes from the endoplasmic reticulum and mitochondrial dysfunction caused by CCl4 administration (Xian *et al*., 2024).

Treatment with LSS in CCl_4-induced liver injury showed an increase in serum albumin levels, total protein, globulin, and A/G ratio, confirming the hepatoprotective effect of LSS (Lakshmi *et al*., 2011). Hepatic damage was also observed with elevated serum total, direct, and indirect bilirubin levels. This increase

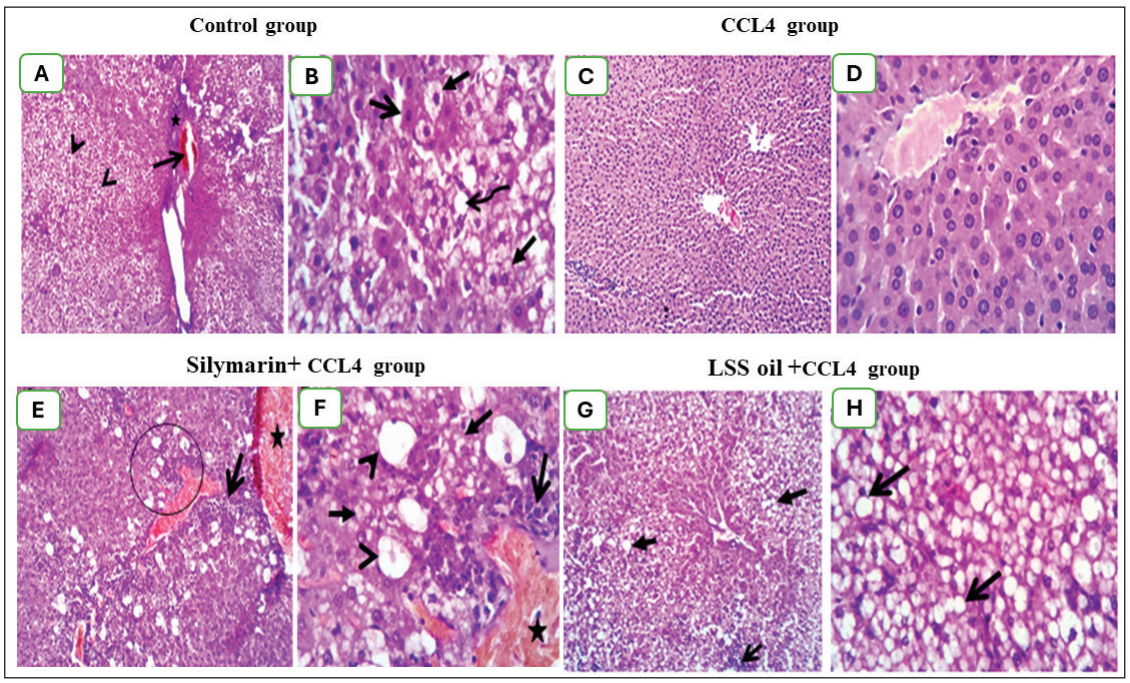

Fig. 14. (A)–(H): Photographs of rat liver section in various treated and control groups. In the control group (14A and 14B), the liver sections of rats showed apparently normal hepatic parenchyma with preserved lobular arrangement, hepatic cords, sinusoids, and vascular tree. Liver section in rats treated with CCL4 treated group (14C) showing ballooning degeneration in a variable number of hepatocytes (arrow heads) beside congestion in portal blood vessels (open arrow) and moderate infiltration of round cells (lymphocytes, macrophages) in portal triads (star, 100X). Moreover, it shows ballooning degenerated cells (closed arrow), signet ring of adipocyte (curved arrow) and apoptotic cells with pyknotic nuclei (open arrow; 14D). The liver of rats in CCL4 + silymarin treated group (14E and 14F), showing vacuolation (closed arrow) mostly macrosteatosis and ballooing changed (arrow heads) with moderate congestion (star) and round cell infiltration in portal triads (open arrow). Lastly, sections from Liver of CCL4 + L.S.S oil treated group (14G, and 14H), showing (14G degenerative changes mostly fatty change in hepatocytes (closed arrows). Interstitial leukocytic infiltration is visible (open arrow), along with micro and macrosteatosis (14H). The first photo in each group was examined at 100X magnification, while the other one was examined at high magnification (400X).

is attributed to impaired bile excretion by the liver, resulting in elevated bile levels in the serum (Elkington *et al*., 1969).

The administration of CCl4 to the animals showed a noticeable increase in total bilirubin (Bishayi *et al*., 2002). Our study reported that treatment with LSS in CCl4-induced liver damage led to a decline in serum total, direct, and indirect bilirubin levels, confirming the potential of LSS to alleviate liver biliary dysfunction. Rats treated with LSS showed a significant reduction in total serum bilirubin compared to those treated with CCl4 alone. The balance of serum total bilirubin and total protein levels through *Lagenaria siceraria* seeds treatment indicates improved liver function (Mani Senthilkumar *et al*., 2005). An extreme concentration of Reactive oxygen species (ROS) and other radicals leads to OS in the body, associated with various pathological conditions. Antioxidants can fight OS and ROS by scavenging free radicals or via their effective reductive capability (Halliwell, 2006). Under pathological conditions, an imbalance between OS and antioxidant defense can lead to oxidative damage in cellular membranes, resulting in lipid peroxidation, DNA degradation, and the formation of oligonucleosomal fragments. Elevated levels of lipid peroxides, such as MDA, can be highly cytotoxic (Halliwell, 2006; Raza *et al*., 2022).

In the extant inquiry, the scavenging of both MDA and supporting the CAT and GSH synthesis indicate the potent antioxidant property of LSS. The significant antioxidant action of the extract, as evidenced by the increase in CAT and GSH levels, suggests its potential protective effect. In rats treated with CCl4, hepatotoxicity occurs due to an imbalance between ROS production and the antioxidant system, leading to cellular damage. This is indicated by the decreased levels of CAT and GSH and a significant reduction in lipid peroxidation in the treated groups, further enhancing its antioxidant activity *in vivo* (Halliwell, 2006; Raja *et al*., 2007).

The protective effect of LSS on liver damage is also evident in histological examinations of hepatic tissue. Histopathological analyses have demonstrated that *Lagenaria siceraria* extract can protect hepatocytes from damage caused by hepatotoxic agents like CCl_4 (Saeed *et al*., 2022). The protective effects of *Lagenaria siceraria* extract on the liver include reduced necrosis, inflammation, fibrosis in liver tissue, and the preservation of normal hepatocyte morphology. The extract's hepatoprotective mechanisms involve scavenging free radicals, modulating antioxidant enzymes, inhibiting inflammatory mediators, and regulating apoptotic pathways in hepatocytes. Bioactive compounds in *Lagenaria siceraria,* such as triterpenoids, flavonoids, and saponins, are believed to contribute to its demonstrated hepatoprotective properties in histopathological studies. Further studies are needed to understand the molecular mechanisms of LSS to improve liver health and other organs.

Conclusion

The study confirmed that LSS has a hepatoprotective effect against CCl_4-induced hepatotoxicity in rats. This protective effect is attributed to the antioxidant capacity of LSS, which reduces MDA levels, promotes CAT and GSH activity, and maintains hepatic enzymes within normal ranges. Additionally, it helps maintain the integrity of liver cells and reduces OS, according to histological examination.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

M.Z. and S.M.E. performed the experiments; N.H.A., S.M.E., and H.M.S. designed the experiments; N.H.A., M.Z., S.M.E., A.K.A., W.E.A., S.G.F., and H.M.S. analyzed data and wrote the initial draft; A.K.A., W.E.A., and S.G.F. revised the manuscript. All authors have read and approved the submission of the final version of this manuscript.

Data availability

All data are provided in the manuscript.

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