Faculty of Medical and Health Sciences, University of Poonch Rawalakot
Journal of Pharma and Biomedics
ISSN: 3007-1984(online), 3007-1976 (Print)
https://www.jpbsci.com/index.php/jpbs

Therapeutic Effect of *Onosma echioides* L. against Hepatocellular Impairment

Nosheen Sadiq[1], Tafail Akbar Mughal[1]*, Sadia Nazer[1], Shazia Khatoon[2], Saeed Khalil[2], Kiran Javaid[1], Iqra Shafique[3], Syed Haseeb ul Hassan Gillani[4], Syeda Sobia Wahab Bukhari[1], Saira Khanum[5], Ayesha Anwar[1]

[1] Department of Zoology, Women University of Azad Jammu & Kashmir, Bagh, Pakistan.
[2] Department of Botany, Women University of Azad Jammu & Kashmir, Bagh, Pakistan.
[3] Azad Jammu and Kashmir Medical College Muzaffarabad, AJ&K, Pakistan.
[4] Pharmacology Department, Akhtar Saeed Medical College Rawalpindi, Pakistan.
[5] Department of Zoology, Mohi-Ud-Din Islamic University Nerian Sharif, AJ&K, Pakistan.

Received: August 30, 2025; *Revised: November 10, 2025;* *Accepted: November 17, 2025*

A B S T R A C T

The study investigates the therapeutic effects of *Onosma echioides* L. extract on hepatocellular impairment induced by carbon tetrachloride (CCl_4) in Swiss albino mice. The CCl_4 (0.4 mL/kg b.w) model was employed to explore liver damage through biochemical, hematological, and histological assessments. Aqueous extracts of *Onosma echioides* (10 g/100 mL) were prepared and administered at low and high doses (200 mg/kg and 400 mg/kg, respectively) to CCl_4 intoxicated mice. Biochemical analyses revealed that CCl_4 significantly elevated liver function markers, including total bilirubin (2.275±0.085 mg/dl), alanine aminotransferase (ALT) (216.75±4.64 /µL), and aspartate aminotransferase (AST) (185±4.24 /µL). Conversely, alkaline phosphatase (ALP) levels (CCl_4: 157.25±7.364 /µL) showed significant decline. Histological analysis indicated significant hepatotoxicity characterized by altered liver morphology and disrupted cellular architecture. Treatment with *Onosma echioides* extract significantly mitigated CCl_4-induced liver damage, as evidenced by normalization of liver function tests and restoration of liver histology. These findings suggest the potential hepatoprotective properties of *Onosma echioides*, highlighting its importance in developing natural therapeutics against liver impairment.

Keywords: *Hepatotoxicity, Onosma echioides, Carbon Tetrachloride, Liver Function Tests, Biochemical Analysis*.

Corresponding Authors: *Tafail Akbar Mughal*
Email: tufail.akbar9@gmail.com

INTRODUCTION

Throughout history, a diverse range of materials has been utilized for the treatment of illnesses and the promotion of health (Alves & Rosa, 2007). Traditional populations worldwide have developed a rich natural pharmacopeia based on the use of wild species, with estimates from the World Health Organization indicating that as many as 80% of people in rural areas of developing countries rely on traditional medicine to meet their primary healthcare needs (Alves & Rosa, 2005). This ongoing practice of using plants and animals in traditional medicine has paved the way for the discovery of numerous natural pharmaceutical compounds that are acknowledged in modern medicine (Kunwar et al., 2008).

Natural products are essential in the treatment of diseases, particularly because plants serve as a primary source of medicinal compounds with their rich variety of secondary metabolites (Atanasov et al., 2015). The therapeutic potential of these bioactive compounds is increasingly recognized, especially in the context of addressing chronic diseases—a significant public health concern. Liver diseases have emerged as a major issue requiring immediate attention, prompting the urgent need for novel hepatoprotective agents (Targher et al., 2021). Metabolic

dysfunction-associated fatty liver disease (MAFLD) and non-alcoholic fatty liver disease (NAFLD) are becoming increasingly prevalent, now affecting approximately 25% of adults globally, and are among the leading causes of liver-related morbidity (Eslam et al., 2022). Furthermore, liver cancer, predominantly hepatocellular carcinoma (HCC), poses a severe health crisis, driven by various risk factors including viral infections, obesity, and environmental toxins such as carbon tetrachloride (CCl_4) (El-Serag & Rudolph, 2007). *Onosma echioides* L., a prominent species in traditional medicine, has garnered attention for its therapeutic benefits, particularly its antioxidant, anti-inflammatory, and hepatoprotective properties (Kumar et al., 2013). CCl4 is frequently employed in experimental models to investigate the mechanisms behind liver damage; its use leads to oxidative stress and subsequent necrosis and inflammation in liver tissue (Mas et al., 2008).

Antioxidants play a crucial role in mitigating oxidative damage and protecting liver cells (Kamel et al., 2010). Recent studies have underscored the importance of natural antioxidants, particularly those obtained from plant sources, in safeguarding against oxidative damage associated with liver disorders (Mas et al., 2008). For example, herbal remedies such as *Hibiscus rosa* have demonstrated significant antioxidant and hepatoprotective capabilities (Abdel-Ghaffar & El-Elaimy, 2012). Collectively, these insights underscore the pressing need for ongoing research into natural compounds that could serve as effective preventative and therapeutic agents for liver diseases. With the increasing prevalence of liver conditions, it is imperative to explore the full potential of traditional medicinal plants, such as *Onosma echioides* L., to contribute meaningful solutions in the fight against hepatic disorders.

MATERIALS AND METHODS

Collection of medicinal plant

Onosma echioides L. plant was taken from local area of Hillan valley, District Haveli Kahutta, Azad Kashmir, Pakistan. To get rid of the dust, the plant roots were washed under running water. The roots were shade dried and crushed into a fine powder.

Figure 1: *Onosma echioides* L. plant root.

Extract preparation

According to earlier study, shade-dried roots bark of *Onosma echioides* L. was utilized to prepare an aqueous extract using maceration (Mughal *et al*., 2020a). 100 ml of distilled water and 10 g of powder were combined, heated, and stirred for an hour. The mixture was filtered using Whatman filter paper after being cooled to room temperature.

Chemicals used

Carbon tetrachloride, chloroform and formaldehyde were obtained from Sigma Aldrich (USA).

Hepatoprotective activity

Swiss albino mice were exposed to liver damage by carbon tetrachloride (CCl_4). By comparing the levels of several biochemical components in plasma, the hepatoprotective efficacy of the *Onosma echioides* L. extract was evaluated.

Animal selection and grouping

This study was limited to the analysis and collection of data from twenty male Swiss albino mice *Musmusculus domesticus* (average age: 8 weeks; body mass: 35±5 g) that were taken from National Institute of Health (NIH) Islamabad. They were accommodated together for seven days afore the commencement of the trial and were divided into four groups; Group I (5 mice): control group; these animals did not get any sort of treatment. Group II (5 mice): CCl_4; Group III (5 mice): CCl_4+Extract (LD) animals treated with oral daily dose (via 18-gauge oral feeding needle) of OE-extract (200 mg/kg) for 15 consective days;

Group IV (5 mice): CCl_4+Extract (HD) animals treated with oral daily dose of OE (400 mg/kg) for 15 consecutive days.

Ethical statement

All trials were carried out in accord with the international laws and University recommendations for the care of experimental animals as mentioned in **(**Mughal et al. 2019; Dar et al. 2019; Ali et al. 2020, Mughal et al., 2020a; Mughal et al., 2020b; Mughal et al., 2022; Nauroze et al., 2023a; Nauroze et al., 2023b; Mughal et al., 2025; Naz et al., 2025**)**.

Glassware and types of equipment used

The following equipment and materials were utilized in the study: Incubator: WP25A Desktop Constant Temperature Incubator; Analytical Balance: SARTORIUS GmbH, Göttingen, Germany; Centrifuge; Digital Weighing Machines; Filter Paper: Whatman® (Schleicher & Schuell Cat No 1442 125); Falcon Tubes: 15 mL; Beakers: 10 mL, 100 mL, 250 mL, 500 mL, and 1000 mL; Conical Flasks: 1000 mL PYREX® IWAKI TE-32; Hot Plates: SCILOGEX MS-H-S; Rotary evaporator, Microscope Slides and Covers and Dissection Box.

Estimations of liver function tests

The AST and ALT activity was determined according to the method of Reitman and Frankel (1957). Appraisal of ALP was done by Bowers method (1966). Total bilirubin level (TBL) was confirmed by modified dimethyl sulfoxide (DMSO) protocol (Dangerfield and Finlayson, 1953).

Hematological assay

After a 14-day treatment period, the mice were sacrificed, and cardiac blood samples were collected from each animal. Blood was drawn into sterile test tubes containing the anticoagulant EDTA. Following the collection, blood samples were centrifuged for 15 minutes at 3000 rpm at room temperature. Hematological parameters, including red blood cells (RBC), white blood cells (WBC), platelets, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were analyzed using the ABC Vet Analyzer. Additionally, a comprehensive blood count (CBC) was performed on the serum samples using the Pentra 400 biochemical analyzer.

Histological studies

At the end of the experiment, liver from the mice was taken for histological analysis. H & E staining was used for histological study.

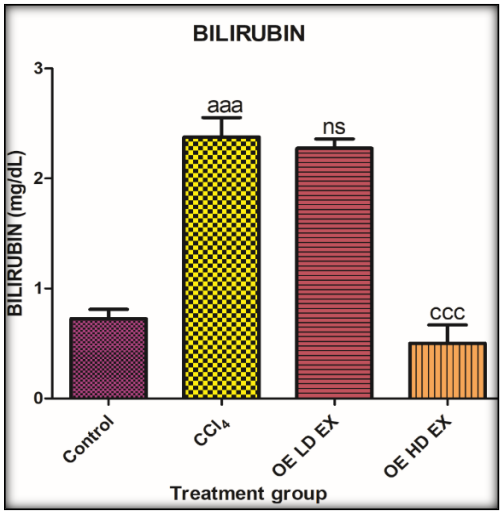
Statistical analysis

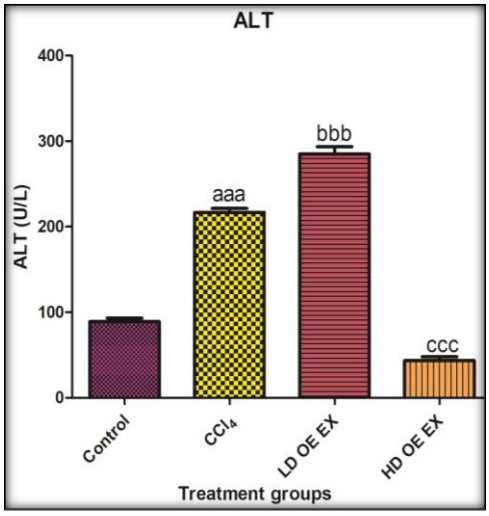
Arithmetic analysis was done by GraphPad prism (version 5.0). The data were presented as Mean±SEM. One way ANOVA with the Bonferroni test was used to assess group differences. The statistical significance level was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect on liver function tests

Administration of carbon tetrachloride (CCl_4) caused a significant increase in total bilirubin, ALT, and AST levels, indicating liver injury, while it significantly decreased ALP levels. Treatment with a high dose of the OE extract significantly reversed the CCl_4-induced elevations in bilirubin, ALT, and AST, bringing their levels down. Conversely, the low dose of OE extract showed no significant effect on bilirubin or AST, but it further increased ALT levels and also caused a significant rise in ALP. The high dose, however, did not produce a significant change in ALP levels compared to the CCl_4-treated group (figure 2).





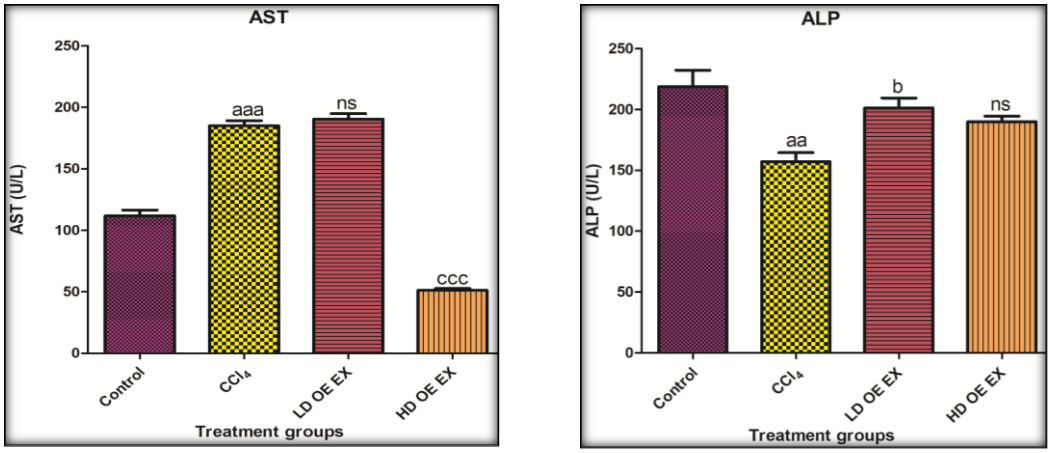
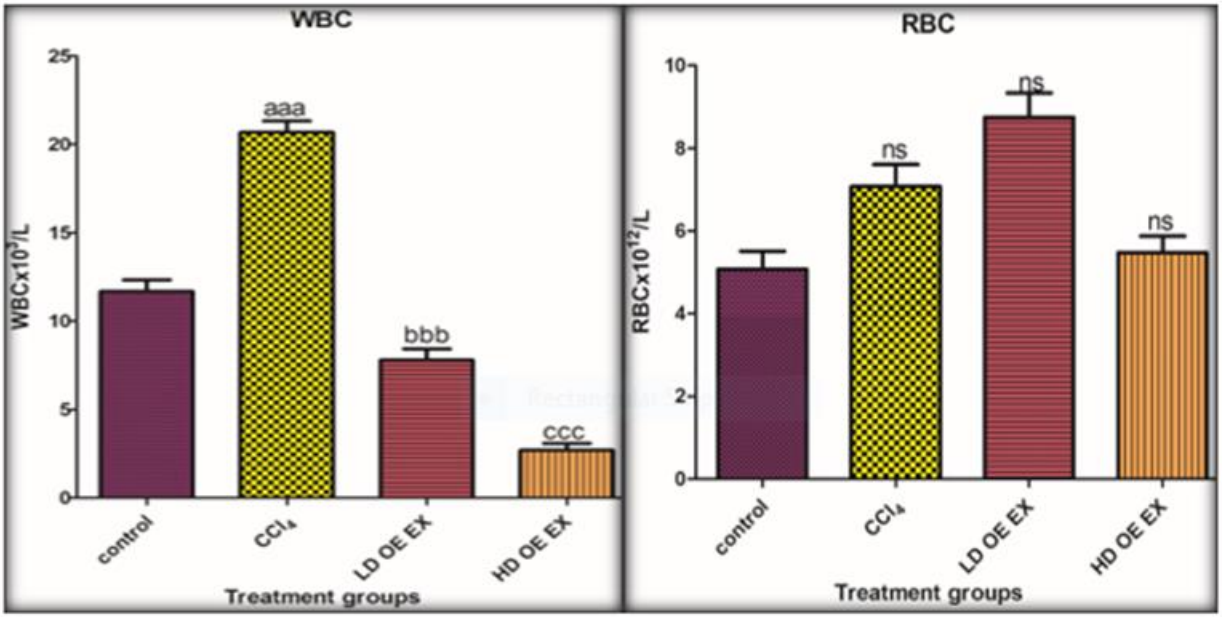


Figure 2: Level of Bilirubin, ALT, AST and ALP. (CCl4: Carbon tetrachloride; OE LD Ex: Onosma echioides low dose extract; OE HD Ex: Onosma echioides high dose extract).

Key:"a" shows significant difference between control and CCl4 treated group. "b" shows significant difference between CCl4 and LD OE Ex treated group. "c" shows significant difference between CCl4 and High dose treated groups. Each bar represents mean value of four replicates and SEM. Statistical icons: b=p≤0.05; aa=p≤0.01; aaa, bbb, ccc=p≤0.001.

Hematology

Based on the hematological analysis, carbon tetrachloride (CCl4) induced a pronounced inflammatory and stress response, marked by a highly significant increase in white blood cells (WBCs) and neutrophils, alongside a severe decrease in lymphocytes. The administration of the OE extract, particularly at a high dose, effectively countered this inflammation, significantly reducing WBC and neutrophil counts while increasing lymphocyte levels back towards normal. CCl4 also caused significant hematological disturbances by increasing platelets and decreasing mean corpuscular volume (MCV) and packed cell volume (PCV). The OE extract treatment demonstrated a corrective effect, significantly increasing MCV at both low and high doses. Notably, the high dose of OE extract also produced a substantial and significant decrease in the elevated platelet count. Most other parameters, including red blood cells, hemoglobin, MCH, and several platelet indices, showed no significant changes in response to either CCl4 or the OE extract treatment (figure 3,4,5).



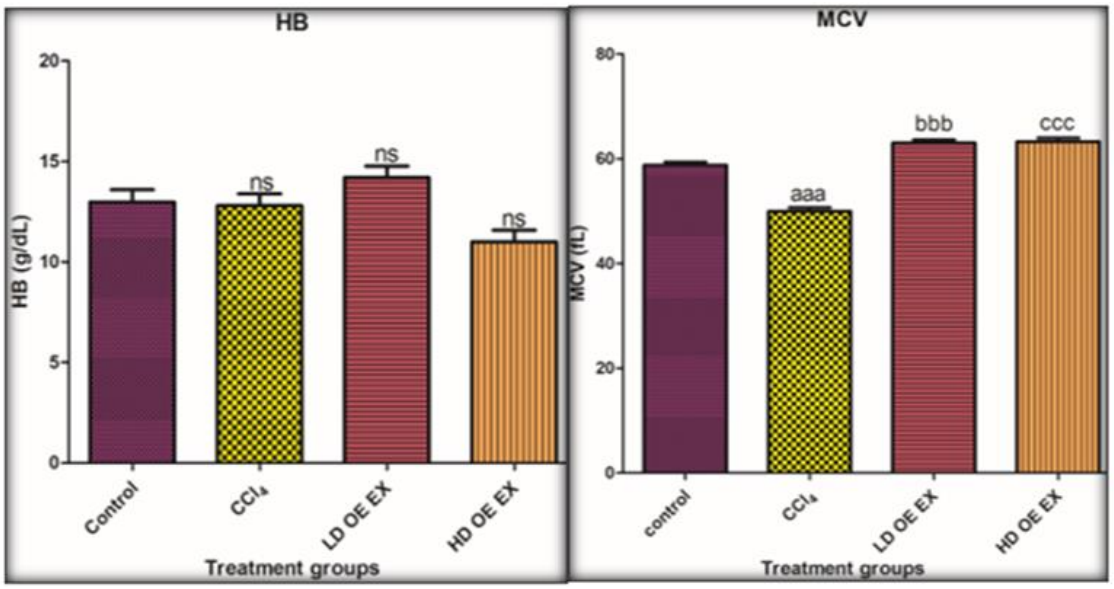


Figure 3: Level of WBCs, RBCs, Hb and MCV.

Key:"a" shows significant difference between control and CCl4 treated group. "b" shows significant difference between CCl4 and LD OE Ex treated group. "c" shows significant difference between CCl4 and High dose treated groups. Each bar represents mean value of four replicates and SEM. Statistical icons: aaa, bbb, ccc=p≤0.001.

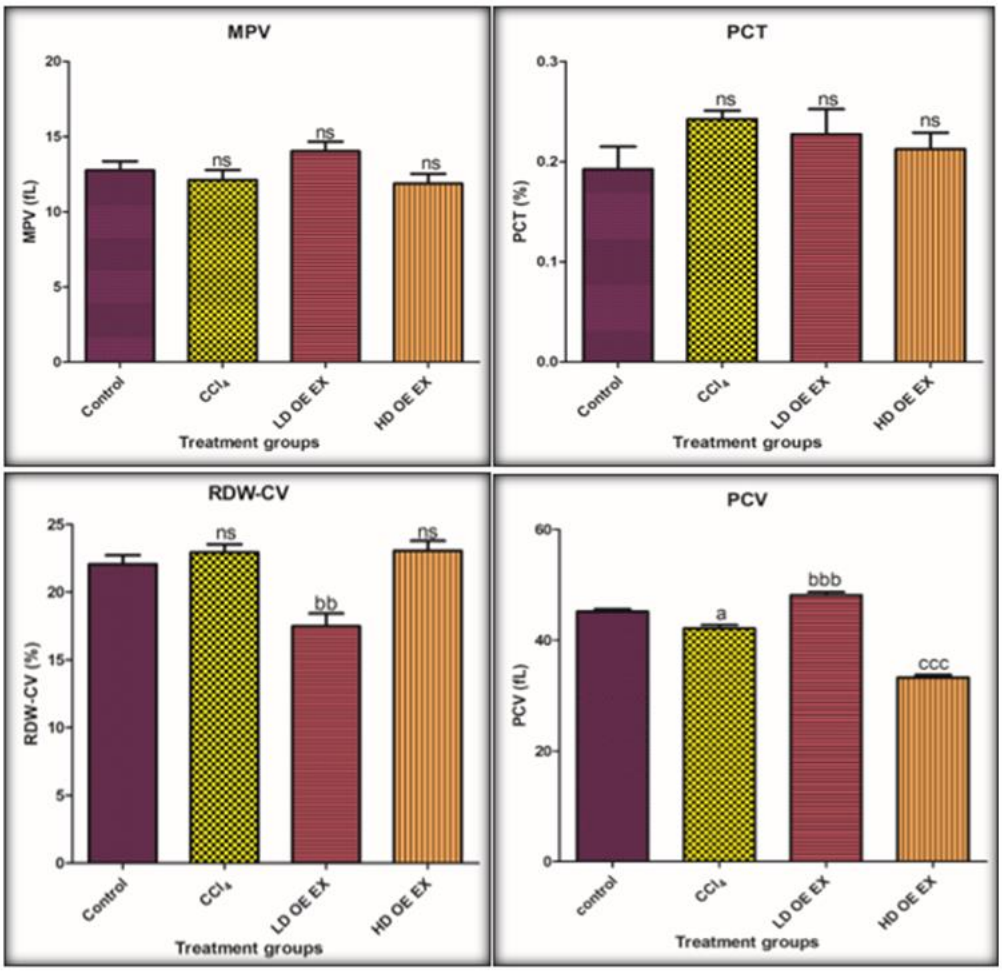


Figure 4: Level of MPV, PCT, RDW-CV and PCV.

Key:“a” shows significant difference between control and CCl4 treated group. “b” shows significant difference between CCl4 and LD OE Ex treated group. “c” shows significant difference between CCl4 and High dose treated groups. Each bar represents mean value of four replicates and SEM. Statistical icons: a=p≤0.05; bb=p≤0.01; bbb, ccc=p≤0.001.

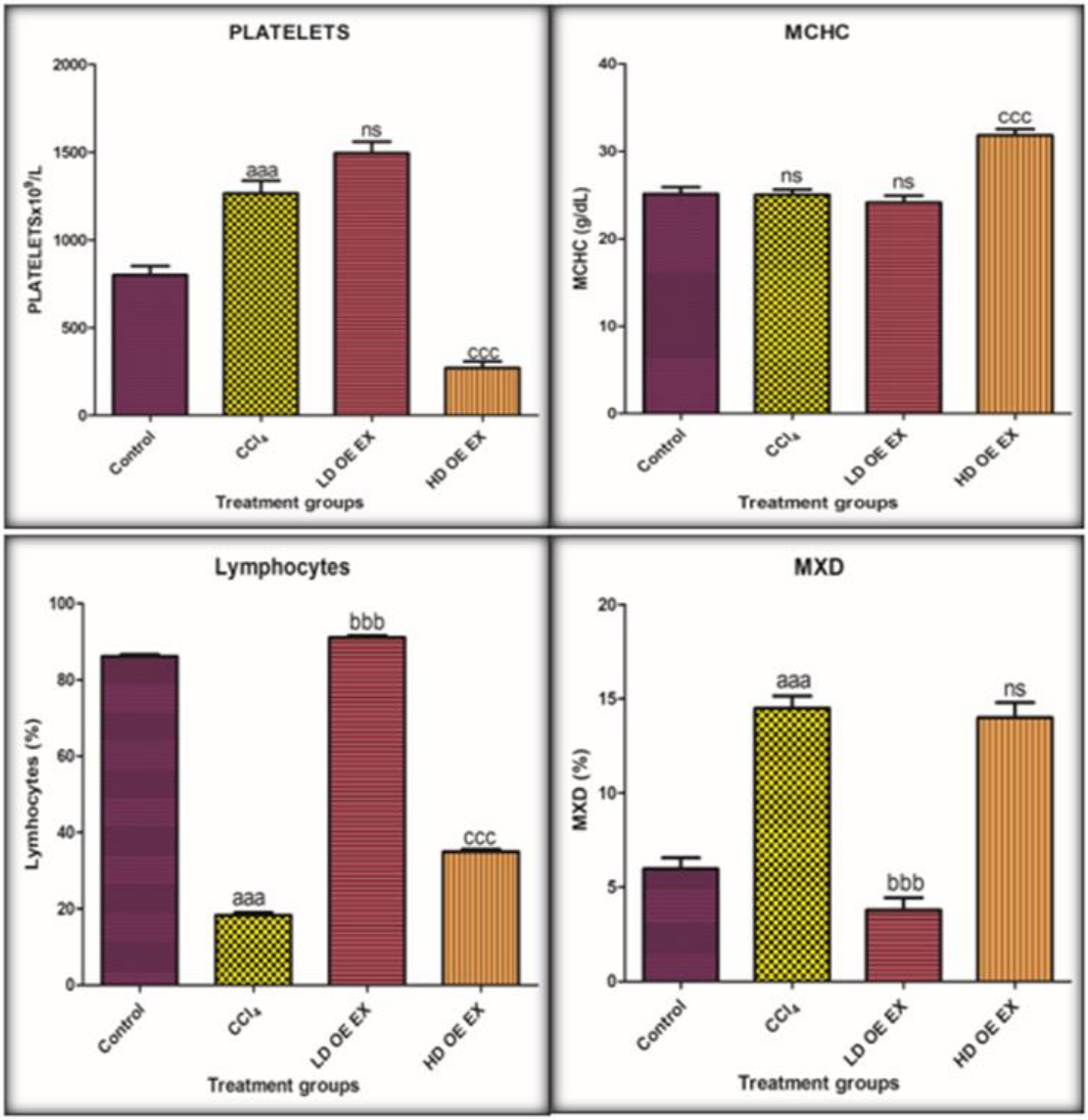


Figure 5: Level of Platelets, MCHC, Lymphocytes and MXD.

Key:“a” shows significant difference between control and CCl4 treated group. “b” shows significant difference between CCl4 and LD OE Ex treated group. “c” shows significant difference between CCl4 and High dose treated groups. Each bar represents mean value of four replicates and SEM. Statistical icons: aaa, bbb, ccc=p≤0.001.

Morphology of liver

Morphology of liver in control group shows normal colour and structure. In case of CCl_4 treated group liver colour and shape was changed. Damage was seen there. But when *Onosma echioides* low dose and high dose extract was given in combination with CCl_4 hepatoprotective effect was shown as there is normal shape and colour was seen in both cases (Figure 6).

Histology

The hepatic section of control group exhibited normal architecture with intact central vein (CV). In CCl_4 treated group, the hepatic showed distressed CV with apoptotic nuclei. *Onosma echioides* low dose and high dose extract treated mice showed significant recovery (Figure 7).

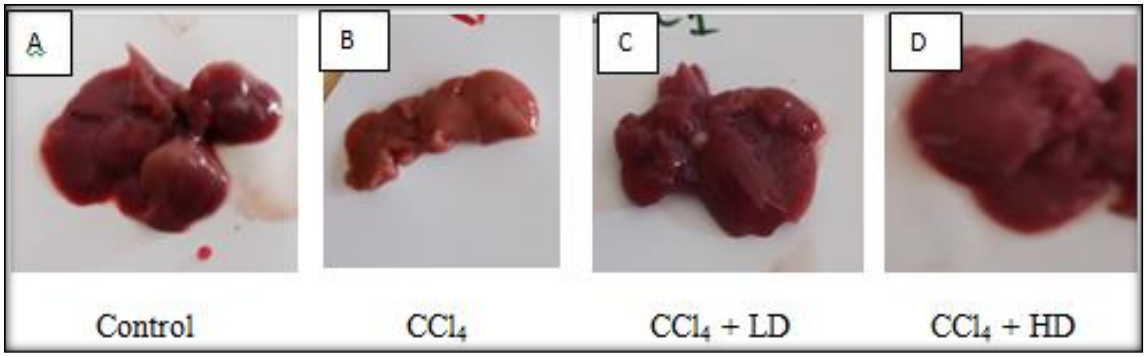


Figure 6: Morphology of liver.

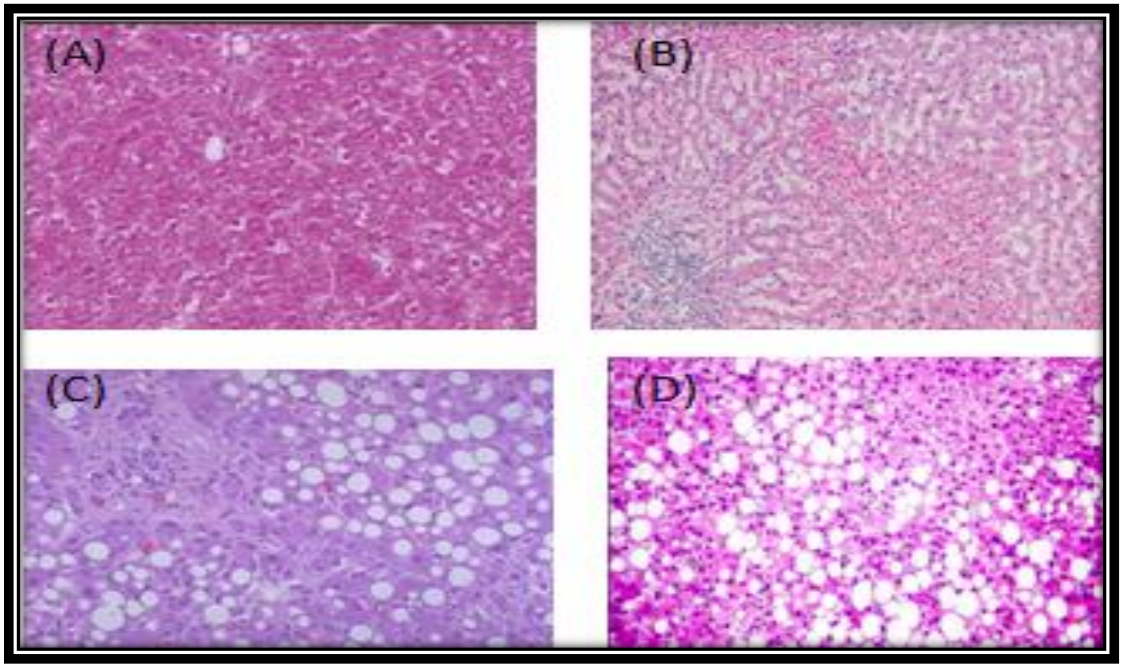


Figure 7: Histology of liver. A. Control B. CCl4 C. CCl4 + LD D. CCl4 + HD

DISSCUSION

The present study highlights the hepatoprotective potential of *Onosma echioides* L. against liver impairment induced by carbon tetrachloride (CCl_4), a widely recognized model for studying hepatotoxicity. The administration of CCl_4 resulted in marked elevations in liver function markers, particularly ALT and AST, indicating hepatocellular damage. These findings are consistent with previous literature, where CCl_4 exposure predominantly leads to oxidative stress and subsequent liver injury (Ritesh et al., 2015; Benahmed et al., 2020). The biochemical assessments revealed a significant increase in total bilirubin levels in CCl_4-treated mice, aligning with the hepatocellular dysfunction typically observed in liver injury. However, treatment with the high dose of *Onosma echioides* extract resulted in a noteworthy reduction in bilirubin levels, which correlates with an improvement in liver function and cellular integrity. This restoration may be attributed to the antioxidant properties of the extract, which combat oxidative stress in the liver and facilitate the detoxification processes (Afzal et al., 2013; Atanasov et al., 2015).

Hematological profiles further corroborated the hepatoprotective effects. CCl_4 administration led to a significant increase in white blood cells (WBCs) and neutrophils, indicative of inflammation and a stress response to tissue damage (Kamel et al., 2010). Notably, the administration of *Onosma echioides* extract significantly decreased WBC and neutrophil counts, reflecting a reduction in inflammation and a potential normalization of the immune response. Moreover, the effect on red blood cell indices (such as MCV) and hemoglobin suggested a possible restoration of hematological health influenced by hepatic function. Interestingly, the extract's high dosage appeared to exacerbate the effects on platelets and

lymphocyte counts, warranting further investigation to elucidate the dose-dependent effects of the extract on hematopoiesis. Histological examinations revealed further validation of the therapeutic effects; CCl_4-treated livers displayed distortions and necrosis, while *Onosma echioides* treatment restored normal hepatic architecture. The integrity of the central vein in treated groups supports the hypothesis that the extract offers a protective effect against CCl_4-induced hepatic degeneration. Such histological improvements correlate with the aforementioned biochemical findings, reinforcing the hepatoprotective implications of *Onosma echioides*. In conclusion, the hepatoprotective effects observed in this study can be attributed to the antioxidant and anti-inflammatory properties of *Onosma echioides*, advocating for its potential as a natural therapeutic agent against liver impairments. Future research should further investigate the active compounds within the extract responsible for these effects and explore their mechanisms of action in depth to facilitate clinical applications in liver disease management.

CONCLUSION

A certain amount of CCl_4 in mice was found to be potentially harmfull to the liver. The damage caused by CCl_4 can be stopped by *Onosma echioides L*. The study showed that *Onosma echioides* L. (LD) significantly increased the levels of number of haematological indices, such as MCV, lymphocytes, PCV, it showed no significant changes in RBC, Hb, platelets, MCH, MCHC, PDW, MPV, PCT, RDW-CV, and it showed significant declines in WBCs, neutrophils and MXD at the current dosages. The *Onosma echioides L.* (HD) significantly increased the levels of number of haematological indices, such as MCV, MCHC, lymphocytes, it showed no significant changes in RBC, HB, MXD, MCH, Platelets, MCH, MCHC, PDW, MPV, PCT, RDW-CV, and it showed significant declines in WBCs, neutrophils, PCV, platelets and MXD at the current dosages. Therefore, the results of the study suggest that *Onosma echioides L.* extract could protect liver against the CCl4 induced oxidative damage in mice.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

Nosheen Sadiq and Tafail Akbar Mughal designed the study. Nosheen sadiq performed experiments. Tafail Akbar Mughal supervised the study. All authors wrote and revised the manuscript.

REFERENCES

Abdel-Ghaffar, F.R. & El-Elaimy, I.A., 2012. *In vitro antioxidant and scavenging activities of Hibiscus rosa-sinensis crude extract*. Journal of Applied Pharmaceutical Science, 2(1), pp.51–58.

Afzal, M. et al., 2013. Comparison of protective and curative potential of *Daucus carota* root extract on renal ischemia reperfusion injury in rats. *Pharmaceutical Biology*, 51(7), pp.856–862.

Ali, S. et al., 2019. Evaluation of chemopreventive and chemotherapeutic effect of *Artemisia vulgaris* extract against diethylnitrosamine-induced hepatocellular carcinogenesis in Balb/C mice. *Brazilian Journal of Biology*, 80, pp.484–496.

Alves, R.R.N. & Rosa, I.L., 2005. Why study the use of animal products in traditional medicines? *Journal of Ethnobiology and Ethnomedicine*, 1, pp.1–5.

Alves, R.R.N., Rosa, I.L. & Santana, G.G., 2007. The role of animal-derived remedies as complementary medicine in Brazil. *BioScience*, 57, pp.949–955.

Atanasov, A.G. et al., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, 33(8), pp.1582–1614.

Benahmed, F. et al., 2020. Protective effect of *Pistacia atlantica* Desf leaves on mercury-induced toxicity in rats. *South Asian Journal of Experimental Biology*, 10(3), pp.152–161.

Bowers, G.N., 1966. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clinical Chemistry*, 12(2), pp.70–89.

Dangerfield, W.G. & Finlayson, R., 1953. Estimation of bilirubin in serum. *Journal of Clinical Pathology*, 6(3), pp.173–177.

Dar, K.K. et al., 2019. In vivo induction of hepatocellular carcinoma by diethylnitrosamine and pharmacological intervention in Balb/C mice using *Bergenia ciliata* extracts. *Brazilian Journal of Biology*, 79(4), pp.629–638.

El-Serag, H.B. & Rudolph, K.L., 2007. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*, 132(7), pp.2557–2576.

Eslam, M. et al., 2022. Metabolic (dysfunction)-associated fatty liver disease in individuals of normal weight. *Nature Reviews Gastroenterology & Hepatology*, 19(10), pp.638–651.

Kamel, H.H., Abd-El-Rahman, H.A., Ahmed, W.M.S. & Mohamed, A.H., 2010. Protective effect of some

antioxidants against CCl_4-induced toxicity in liver cells from BRL3A cell line. *Journal of American Science*, 6, pp.992–1003.

Kumar, N., Kumar, R. & Kishore, K., 2013. *Onosma* L.: A review of phytochemistry and ethnopharmacology. *Pharmacognosy Reviews*, 7(14), pp.140–152.

Kunwar, R. & Bussmann, R.W., 2008. Ethnobotany in the Nepal Himalaya. *Journal of Ethnobiology and Ethnomedicine*, 4, p.24.

Mas, N. et al., 2008. Ursodeoxycholic acid treatment improves hepatocyte ultrastructure in rat liver fibrosis. *World Journal of Gastroenterology*, 14, pp.1108–1111.

Mughal, T.A. et al., 2020a. Carbon tetrachloride-induced hepatocellular damage in Balb/C mice and pharmacological intervention by extract of *Daucus carota*. *RADS Journal of Pharmacy and Pharmaceutical Sciences*, 8(4), p.1.

Mughal, T.A. et al., 2019. Evaluation of hepatotoxicity of carbon tetrachloride and pharmacological intervention by vitamin E in Balb/C mice. *Pakistan Journal of Zoology*, 51(2), pp.755–761.

Mughal, T.A. et al., 2025. Bioformation of silver nanoparticles from *Berberis lyceum* Royle and their application as hepatoprotective agent. *Planta Animalia*, 4(4), pp.421–431.

Mughal, T.A. et al., 2022. Phytochemical screening, antimicrobial activity, in vitro and in vivo antioxidant activity of *Berberis lycium* Royle root bark extract. *Brazilian Journal of Biology*, 84, p.e249742.

Mughal, T.A. et al., 2020b. Anti-hyperlipidemic effect of *Berberis lyceum* Royle root bark extract in alloxanized Swiss albino mice. *Punjab University Journal of Zoology*, 35(2), pp.261–268.

Nauroze, T. et al., 2023a. Ameliorative effect of *Nigella sativa*-conjugated silver nanoparticles against chromium-induced hepatotoxicity and renal toxicity in mice. *Saudi Journal of Biological Sciences*, 30(3), p.103571.

Nauroze, T. et al., 2023b. Pharmacological intervention of biosynthesized *Nigella sativa* silver nanoparticles against hexavalent chromium–induced toxicity in male albino mice. *Saudi Journal of Biological Sciences*, 30(3), p.103570.

Naz, F. et al., 2025. Biogenic silver nanoparticles from *Labeo rohita* scales: antibacterial, antioxidant, antibiofilm and synergistic activities. *Planta Animalia*, 4(5), pp.355–365.

Reitman, S. & Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1), pp.56–63.

Ritesh, K.R. et al., 2015. A single acute hepatotoxic dose of CCl_4 causes oxidative stress in the rat brain. *Toxicology Reports*, 2, pp.891–895.

Targher, G., Tilg, H. & Byrne, C.D., 2021. Non-alcoholic fatty liver disease: a multisystem disease requiring a multidisciplinary and holistic approach. *The Lancet Gastroenterology & Hepatology*, 6(7), pp.578–588.