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Nature's Defense: *Elaegnus umbellata* as a Potent Ally Against Carbon Tetrachloride-Induced Liver Injury

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Abstract

Background: Hepatocellular damage in liver failure depends on type, duration and severity of toxic agent. The accumulation of toxins enhances liver damage leading to hepatocellular apoptosis and necrosis. It can lead to scarring (cirrhosis), which is a life threatening condition**.** Many plants are used as protective agent against hepatocellular impairment. The current study aims to investigate the preventative effects of *Elaeagnus umbellata* fruit extract on carbon tetra chloride induced hepatocellular injury in mice. **Methods:** Four groups of twenty male swiss albino mice were made: the control group, the CCl_4 group, the low dose extract treated group (CCl_4+ low dose), and the high dose extract treated group (CCl_4+ high dose). **Result:** Higher plasma concentrations comprising the enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) at the end of the experiment indicated hepatocyte apoptosis and necrosis as well as portal/periportal inflammation in the group treated with CCl_4. In contrast, treatment of *Elaeagnus umbellata* extract at low and high doses effectively reduced the increased AST, ALP, and ALT activity in plasma. Liver histopathology showed that *Elaeagnus umbellata* extract reduced the occurrence of liver abnormalities in mice. **Conclusion:** *Elaeagnus umbellata* has potential to shield liver from oxidative damage.

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INTRODUCTION

The liver, which accounts for more than half of an adult total body weight and weighs between 1,200 and 1,500 g, in the human body and is the largest solid organ (Dooley et al., 2018). In addition to many other functions, it helps in the breakdown and removal of poisons, including drugs and other foreign chemicals and serves as the principal location for poisons absorbed from the digestive tract (Okaiyeto et al., 2018; Mughal et al., 2019; Mughal et al., 2020; Mughal et al., 2024). The liver is where the cytochrome P-450 family of enzymes largely metabolise drugs (Okaiyeto et al., 2018; Mughal et al., 2019; Mughal et al., 2020; Mughal et al., 2024). The liver has a high metabolic rate and one of the most abundant populations of mitochondria (An et al., 2020). Each hepatocyte contains between 1000 and 2000 mitochondria, or around 18% the overall volume (An et al., 2020).

CCl_4 is rapidly taken into the body by eating, breathing, and cutaneous absorption. The majority of human lung absorption is assumed to be accounted for by the primary exposure mechanism, breathing. The pace at which chemicals are absorbed from the digestive tract is significantly influenced by the meal; for example, alcohol or fat

can hasten the intestinal absorption of CCl_4 (Weber et al., 2003).

CCl_4 causes problems with the brain, testicles, kidneys, and lungs. Through metabolic activation with highly reactive compounds like free radicals, some chemicals, including numerous environmental pollutants and clinically beneficial medications, can dramatically damage cells in many of our body's organs (Kalantari et al., 2018). Oxidative trauma in the liver is caused by CCl_4. The imbalance between oxidation and antioxidant systems results in the generation of too many free radicals, reduction in antioxidant capacity, is the process behind liver damage that is caused by oxidative stress (Lee et al., 2019; Mughal et al., 2024a).

It is fine identified that CCl_4 causes the creation of oxygen free radicals in a assortment of tissues, comprising the blood, brain, kidney, and liver. The cytochrome oxidase enzyme complex breaks down CCl_4 into trichloromethyl peroxyl ($Cl_3COO•$) and trichloromethyl ($CCl_3•$) radicals. Cytoplasmic membrane phospholipids experience lipid peroxidation as a result of these free radicals. The cell membrane undergoes observable alterations as a result of lipid peroxidation (Van der Paal et al., 2016). CCl_4 is a strong protein- and lipid-bound hepatotoxin that is used to accelerate the peroxidative process (Nazir et al., 2021). Its hazardous effects depend on the trichloromethyl peroxyl radical's ($CCl_3•$) excessive generation (Lina et al., 2017). Reactive oxygen species (ROS) can form from it in a diversity of dynamic tissues, counting the blood, brain, kidney, and liver (Ayala et al., 2014). Toxic free radicals cause discernible lipid peroxidation, which impairs cell membranes disproportionately and causes an increase in a quantity of extreme deviations in renal injury (Sharma, 2015).

The fruit/berry is a great source of important fatty acids, minerals, flavonoids, alkaloids, steroids, terpenoids, and other nutrients like terpenoids and saponins (Bhuvaneswari et al., 2005; Wu et al., 2011; Perveen et al., 2015; Patel, 2015). The fruits of Elaeagnus plant contain large amounts of the phenolic acids (cinnamic and benzoic acids) and flavonoids (epigallocatechin gallate and myricetin). The fruits and barriers of the Elaeagnus plant also include a number of beneficial compounds, such as lutein, phytofluene, phytoene, alpha-carotene, beta-cryptoxanthin, and beta-cryptoxanthin (Patel, 2015).

MATERIALS AND METHODS

Collection of sample

Elaeagnus umbellata was collected from Rawalakot. It was identified by ethno-botanist, department of Botany, Women University of Azad Jammu and Kashmir, Bagh. The fruit of *Elaeagnus umbellata* dried under shade at room temperature. The dried fruits were grinded into fine powder. The current study was related to hepatoprotective activity (liver functional test, hematological assay and histopathological studies).

Preparation of Extract

Fine powder was used for the preparation of aqueous extract according to a standard protocol of maceration (Mughal et al., 2019; Mughal et al., 2020a; Mughal et al., 2020b; Mughal et al., 2022; Mughal et al., 2024a,b). Approximately 10 gram of powder was added to distill water.

Animal Selection and Grouping

The National Institute of Health (NIH) Islamabad provided twenty male swiss albino mice (*Musmusculus domesticus*; average age 8 weeks; body mass 35 g) for the purpose of this study. Before the trial began, they were housed together for fourteen days before being divided into four groups in a unique method. Group I was the control group, which received no therapy at all. Group II: CCl_4 induced group (0.4 ml/kg b.w.). Group III: CCl_4 + Eu low (200 mg/kg b.w) dose. Group IV: CCl_4 + Eu high dose (400 mg/ kg b.w.). Animals were treated with oral daily dose of Eu extract for 14 consecutive days.

Ethics Approval

Animal trials were carried in accordance with international law (Wet op de dierproeven, Wod, Article 9 of Dutch Law as mentioned in our former studies (Mughal et al. 2019; Mughal et al., 2020a; Mughal et al., 2020b; Mughal et al., 2022; Nauroze et al., 2023a; Nauroze et al., 2023b; Mughal et al., 2023; Mughal et al., 2024a,b).

Hepatoprotective activity

Hepatocellular damage was caused in swiss albino mice by induction of CCl_4. The learning's impartial was to evaluate the effesctiveness of by *Elaeagnus umbellata* to treat hepatocellular injury.

Haematological assays, histopathology analyses, and LFTs were the tests conducted.

Liver function test

Liver biomarkers alkaline phosphatase (ALP), bilirubin, alanine transaminase (ALT), aspartate transaminase (ATS) were tested as mentioned in Mughal et al., (2024a) and Mughal et al., (2024b).

Hematological Assay

Mice were killed at the completion of the experiment, and blood samples were collected and tested for complete blood count (CBC) as mentioned in Mughal et al., 2024b.

Histopathological studies

All mice's livers were collected, and histopathology was performed by H & E as mentioned in Mughal et al., 2024b.

Statistical analysis

Graph pad prism (version 5.0) was used for the statistical analysis. All the data were presented as Mean±SEM. One way ANOVA with the Bonferroni test was used to analyse the difference between the groups. The level of significance for statistical analysis was set at $p \leq 0.05$.

RESULTS

Liver function test

For the evaluation of protective effect of Eu-extract against CCl_4 induced hepato-toxicity level of bilirubin, ALT, AST and ALP was measured.

Effect on Bilirubin

IP injection of CCl_4 (0.4 ml/kg b.w.) triggered a considerable increase in bilirubin levels (2.54±0.10 mg/dl) vs the control (0.6±0.15 mg/dl). Mice treated with CCl_4 received a low dose of Eu extract, which resulted in a considerable drop in bilirubin levels (1.2±0.14 mg/dl) matched to the group treated with CCl_4 (2.54±0.10) and when mice were given a large dose of Eu extract, a significant reduction was once more seen (0.91±0.03 mg/dl) as compared to CCl_4 treated group (2.54±0.10) (Figure 2a).

Effect on Alanine Transaminase (ALT)

IP injection of CCl_4 (0.4 ml/kg b.w.) instigated a considerable increase in ALT levels (207.8±5.53 u/L) vs the control (94±4.40 u/L). When low dose of Eu extract was given to CCl_4 treated mice, vast significant decline in level of ALT (78.4±5.64 u/L) was observed as compared to CCl_4 treated group (207.8±5.53) and when high dose of Eu extract was given to mice significant decline was again observed (34±2.02 u/L) as compared to CCl_4 treated group (207.8±5. 53) (Figure 2b).

Effect on Aspartate amino transfarase (AST)

IP injection of CCl_4 (0.4 ml/kg b.w.) caused a substantial increase in AST levels (185.8±3.63 u/L) as compared to control (110±3.53 u/L). When low dose of Eu extract was given to CCl_4 treated mice, vast significant decline in level of AST (58.2±4.223 u/L) compared to the group treated with CCl_4 (185.8±3.63) and when high dose of Eu extract was given to mice significant decline was again observed (57.2±2.51 u/L) as compared to CCl_4 treated group (185.8±3.63) (Figure 2c).

Effect on Alkaline phosphatase (ALP)

0.4 ml/kg b.w. of CCl_4 intraperitoneally (IP) induced a dramatic decrease in ALP levels (172.4±3.63 u/L) vs the control (235.2±3.39 u/L). Mice treated with CCl_4 received a low dose of Eu extract, which resulted in a considerable drop in ALP levels (145±3.53 u/L) was observed as compared to CCl_4 treated group (172.4±3.63) and a considerable rise was seen when mice were given a large dose of Eu extract (184.6±2.51 u/L) vs the CCl_4 treated group (172.4±3.63) (Figure 2d).

Hematology

White blood cells(WBCs), red blood cells (RBCs), Hemoglobin, PCV, MCV, MC, MCHC, Plateletcount, Lymphocytes, MXD, Neutrophils, RDW-CV, PDW, MPV, PCT were calculated in all groups (Eigure 3).

White Blood Cells (WBCs)

0.4 ml/kg b.w. of CCl_4 intraperitoneally (IP) induced a highly major increase in WBC counts (20.16±0.61 $x10^3$/ml) as compared to control (11.4±0.32 $x10^3$/ml). When low dose of Eu extract was given to CCl_4 treated mice, vast significant decline in level of WBCs (7.96±0.47 $x10^3$/ml) was observed as associated to CCl_4 treated group (20.16±0.61 $x10^3$/ml) and when high dose of Eu extract was given to mice significant decline was observed (9.96±0.50 $x10^3$/ml) as compared to CCl_4 treated group (20.16±0.61 $x10^3$/ml).

Red Blood Cells (RBCs)

A 0.4 ml/kg b.w. IP injection of CCl_4 did not significantly alter the RBC levels (8.38±0.74 $x10^{12}$/L) as equated to control (6.9±0.51 $x10^{12}$/L). No discernible difference in RBC level was seen when mice treated with CCl_4 were given a modest dosage of Eu extract (7.8±0.56 $x10^{12}$/L) compared

to the group treated with CCl_4 (8.38±0.74) and when mice were given a large dose of Eu extract, no visible difference was seen (7.06±0.60 $x10^{12}$/L) as compared to CCl_4 treated group (8.38±0.74).

Haemoglobin (Hb)

A 0.4 ml/kg b.w. IP injection of CCl_4 did not significantly alter the levels of HB (13±1.14 g/dl) as compared to control (13.36±0.58 g/dl). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of HB (12.1±0.49 g/dl) was observed as compared to CCl_4 treated group (13±1.14) and when high dose of Eu extract was given to mice no significant difference was observed (11.68±0.88 g/dl) as compared to CCl_4 treated group (13±1.14).

Packed Cell Volume (PCV)

No visible difference in PCV levels was caused by an IP injection of CCl_4 (0.4 ml/kg b.w.) (43.32±0.96 %) as compared to control (46.2±0.61 %). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of PCV (37.1±8.26 %) was observed as compared to CCl_4 treated group (43.32±0.96 %) and when high dose of Eu extract was given to mice no significant difference was observed (34.46±1.09 %) as compared to CCl_4 treated group (43.32±0.96 %).

Mean Corpuscular Volume (MCV)

IP injection of CCl_4 (0.4 ml/kg b.w.) caused a dramatic decrease in MCV levels (50.56±1.10 fl) as compared to control (57.64±0.95 fl). When low dose of Eu extract was given to CCl_4 treated mice, significant upsurge in level of MCV (70.08±0.64 fl) was observed as compared to CCl_4 treated group (50.56±1.10 fl) and when high dose of Eu extract was given to mice significant increase was observed (60.58±0.71 fl) as compared to CCl_4 treated group (50.56±1.10 fl).

Mean Corpuscular hemoglobin (MCH)

No discernible variation in MCH levels was induced by an IP injection of CCl_4 (0.4 ml/kg b.w.) (17.08±0.49 Pg/cell) as compared to control (19.22±0.75 Pg/cell). No discernible difference in MCH level was seen when mice treated with CCl_4 were given a modest dosage of Eu extract (19.16±0.66 Pg/cell) was observed as compared to CCl_4 treated group (17.08±0.49 pg/cell) and when high dose of Eu extract was given to mice no significant difference was observed (15.04±1.05 Pg/cell) as compared to CCl_4 treated group (17.08±0.49 pg/cell).

Mean Corpuscular hemoglobin concentration (MCHC)

A 0.4 ml/kg b.w. IP injection of CCl_4 did not significantly alter the levels of MCHC (25.3±0.79 g/dL) as linked to control (25.06±0.98 g/dL). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of MCHC (28.2±0.75 g/dL) was observed as associated to CCl_4 treated group (25.3±0.79 g/dL) and when high dose of Eu extract was given to mice no significant difference observed (23.24±0.91 g/dL) as matched to CCl_4 treated group (25.3±0.79 g/dL).

Platelets

IP injection of CCl_4 (0.4 ml/kg b.w.) triggered a considerable increase in platelet counts (1309.8±2.26 $x10^9$/L) as compared to control (835.6±3.41 $x10^9$/L). When low dose of Eu extract was given to CCl_4 treated mice, significant decline in level of platelets (1194.2±2.28 $x10^9$/L) was observed as compared to CCl_4 treated group (1309.8±2.26 10^9/L) and when high dose of Eu extract was given to mice significant decline was observed (536±3.20 $x10^9$/L) as compared to CCl_4 treated group (1309.8±2.26 10^9/L).

Lymphocytes

IP injection of CCl_4 (0.4 ml/kg b.w.) instigated a dramatic decrease in lymphocyte counts (19.04±0.56 %) as compared to control (86.16±0.77 %). When low dose of Eu extract was given to CCl_4 treated mice, vast significant upsurge in level of Lymphocytes (59.18±0.74 %) was observed as linked to CCl_4 treated group (19.04±0.56) and when high dose of Eu extract was given to mice significant increase was observed (78.04±0.80 %) as compared to CCl_4 treated group (19.04±0.56).

Mixed Cell Count (MXD)

IP injection of CCl_4 (0.4 ml/kg b.w.) triggered a dramatic increase in MXD levels (15.4±1.36 %) as related to control (6.9±0.50 %). When low dose of Eu extract was given to CCl_4 treated mice, significant decline in level of MXD (8.9±0.48 %) was observed as compared to CCl_4 treated group (15.4±1.36) and when high dose of Eu extract was given to mice significant decline was observed (8.5±0.66 %) as compared to CCl_4 treated group (15.4±1.36).

Neutrophils

CCl_4 (0.4 ml/kg b.w.) intraperitoneally induced a marked increase in neutrophil counts (65.72±0.84 %) as compared to control (9.24±0.76 %). When low dose of Eu extract was given to CCl_4 treated mice, significant decline in level of Neutrophils (31.82±0.87 %) was observed as associated to CCl_4 treated group (65.72±0.84) and when high dose of Eu extract was given to mice significant decline was observed (12.58±0.66 %) as compared to CCl_4 treated group (65.72±0.84).

Red Cell distribution Width (RDW)

A 0.4 ml/kg b.w. IP injection of CCl_4 did not significantly alter RDW levels (22.46±1.17 %) as compared to control (23.14±0.86 %). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of RDW (19.76±0.9 %) was observed as compared to CCl_4 treated group (22.46±1.17) and when high dose of Eu extract was given to mice no significant difference was observed (19.06±1.08 %) as compared to CCl_4 treated group (22.46±1.17).

Platelet distribution Width (PDW)

IP injection of CCl_4 (0.4 ml/kg b.w) instigated no significant differenc in levels of PDW (16.44±1.17 fl) as compared to control (15.24±0.80 fl). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of PDW (18.86±1.11 fl) was observed as compared to CCl_4 treated group (16.44±1.17 fl) and when high dose of Eu extrasct was given to mice no significant difference was observed (14.3±0.94 fl) as compared to CCl_4 treated group (16.44±1.17 fl).

Mean platelet volume (MPV)

No discernible difference in MPV levels was caused by an IP injection of CCl_4 (0.4 ml/kg b.w.) (12.3±0.63 fl) as compared to control (12.06±0.83 fl). When low dose of Eu extract was given to CCl_4 treated mice, significant upsurge in level of MPV (16.14±1.06 fl) was observed as matched to CCl_4 treated group (12.3±0.63 fl) and when high dose of Eu extract was given to mice no significant difference was observed (12.28±0.51 fl) as compared to CCl_4 treated group (12.3±0.63 fl).

Procalcitonin test (PCT)

No discernible variation in PCT levels was prompted by an IP injection of CCl_4 (0.4 ml/kg b.w.) (0.0254±0.01 ng/ml) as compared to control (0.228±0.01 ng/ml). When low dose of Eu extract was given to CCl_4 treated mice, no significant difference in level of PCT (0.0218±0.01 ng/ml) was observed as linked to CCl_4 treated group (0.0254±0.01 ng/ml) and when high dose of Eu extract was given to mice no significant difference was observed (0.284±0.01 ng/ml) as compared to CCl_4 treated group (0.0254±0.01 ng/ml).

Histopathological Studies

According to a histopathological analysis, the case in the control group, the central vein often located in the core of the lobules (Fig. 4A). Central vein was harmed by intraperitoneal CCl_4 injection. In the group treated with CCl_4, the central vein is dilated and causes leisons (Fig. 4B). In mice, liver lesions such as lymphocyte infiltration, hepatic necrosis and the growth of fibrous connective tissue were the results of by CCl_4 were reduced by low and high doses of *Elaeagnus umbellata* extract, according to liver histology (Fig. 4C, 4D). As a product, the verdicts of this study imply that *Elaeagnus umbellata* extract could shield mice's livers from oxidative damage brought on by CCl_4.

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DISCUSSION

As a refrigerant, both a dry cleaning agent and an oil and fats solvent, carbon tetrachloride (CCl_4) serves these purposes. Its vapours can cause liver and kidney deterioration and can also lower central nervous system function. CCl_4 has been shown to cause cancer in experimental animals, therefore it is reasonable to assume that it will do the same in humans. The kidneys and liver are harmed when it is inhaled (Ritesh et al., 2015). The liver serves as our body's primary detoxifying organ, hence it is responsible for the majority of toxicological issues (Rane et al., 2016; Mughal et al., 2019; Mughal et al., 2020; Mughal et al., 2024). Hepatotoxicants cause oxidative damage to liver cells (Singh et al., 2016). This shows the importance of researching CCl_4 -induced hepatotoxicity and potential preventive measures. There has to be more research done to determine the mechanism of liver damage.

In current research level of ALT, AST and bilirubin increased in CCl_4 treated group while level of ALP was decreased significantly. These results are in line with some previous studies (Mughal et al., 2019; Mughal et al., 2020; Mughal et al., 2024). It is well acknowledged that when CCl_4 arrives hepatocytes, it creates free radicals that lead to

peroxidation, which causes the structure of the liver to be destroyed (Xu et al., 2019). Damaged liver cells go through degeneration and necrosis, which causes the release of ALT and AST that are deposited into the blood (Cao et al., 2015). An increase in serum levels of liver enzymes is the primary effect of liver injury (Darwish et al., 2013). Contrarily, exposure to hepatotoxic substances causes liver parenchymal damage as well as an upsurge in plasma bilirubin levels (Darbar et al., 2011). This can be explained by hemolysis caused by reactive species attacking the erythrocyte membrane or bile duct injury, which then results in elevated bilirubin levels (Kalaidjieva and Iliev, 2000). These CCl_4-induced changes were significantly reversed when effected mice were treated with E. umbellata extract which may be because of the capacity of phyto-constituents to maintain cell recovery in liver, in this way ensuring membrane integrity, along with diminishing enzymatic leakage (Jadon et al., 2007).

Swiss albino mice were given a single dose of CCl_4 intraperitoneally, which caused significant increase in WBCs, platelets, neutrophils and MXD, while a significant decrease in lymphocytes and MCV was seen when compared to control. The contents of Hb, RBCs, MCH, MCHC, PCT, and RDW were considerably unchanged when *E. umbellata* extract was administered in combination with it.

Central vein was harmed by intraperitoneal CCl_4 injection. In the group treated with CCl_4, the central vein was dilated and caused leisons. According to liver histology, *E. umbellata* extract, both at low and high doses, maintained the normal arcitecture.

CONCLUSION

Elaeagnus umbellata has potential to shield liver from oxidative damage caused by CCl_4.

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Statement of conflict of interest

None to declare.

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REFERENCES

An, P., Wei, L. L., Zhao, S., Sverdlov, D. Y., Vaid, K. A., Miyamoto, M., & Popov, Y. V. (2020). Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis. Nature communications, 11(1), 1-15.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity, 2014(1), 360438.

Bhuvaneswari, V., & Nagini, S. (2005). Lycopene: a review of its potential as an anticancer agent. Current Medicinal Chemistry-Anti-Cancer Agents, 5(6), 627-635.

Cao, Y. W., Jiang, Y., Zhang, D. Y., Wang, M., Chen, W. S., Su, H. ... & Wan, J. B. (2015). Protective effects of Penthorum chinense Pursh against chronic ethanol-induced liver injury in mice. Journal of Ethnopharmacology, 161, 92-98.

Darbar, S. O. U. M. E. N. D. R. A., Bhattacharya, A. B. H. I. J. I. T., & Chattopadhyay, S. H. Y. A. M. A. P. R. A. S. A. D. (2011). Antihepatoprotective potential of livina, a polyherbal preparation on paracetamol induced hepatotoxicity: A comparison with silymarin. Asian Journal of Pharmaceutical and Clinical Research, 4(1), 72-77.

Darwish, M. M., & Abd El Azime, A. S. (2013). Role of cardamom (*Elettaria cardamomum*) in ameliorating radiation induced oxidative stress in rats. Arab Journal of Nuclear Sciences and Applications, 46(1), 232-239.

Dooley, J. S., Lok, A. S., Garcia-Tsao, G., & Pinzani, M. (Eds.). (2018). Sherlock's diseases of the liver and biliary system. John Wiley & Sons.

Doudach, L., Omari, N. E., Mrabti, H. N., Touhami, F., Mrabti, N. N., Benrahou, K. ... & Faouzi, M. E. A. (2022). Hepatoprotective Effect of *Corrigiola Telephiifolia* Pourr. Root Methanolic Extracts against CCl_4-Induced Hepatic Damage in

Mice. Biointerface Research in Applied Chemistry, 12(2), 2489-2502.

Jadon, A., Bhadauria, M., & Shukla, S. (2007). Protective effect of Terminalia belerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. Journal of ethnopharmacology, 109(2), 214-218.

Kalaidjieva, V. C., & Iliev, Z. K. (2000). Plasma erythropoietin level in rats after kidney proximal tubular impairment. Folia Medica, 42(3), 41-45.

Kalantari, H., Pajou, M. D., Kheradmand, P., Goodarzian, M., & Zeidooni, L. (2018). Nephroprotective effect of hydroalcoholic extract *Allium jesdianum boiss* against carbon tetrachloride induced nephrotoxicity via stress oxidative in mice. Pharmaceutical Sciences, 24(2), 89-96.

Lee, H. Y., Lee, G. H., Yoon, Y., & Chae, H. J. (2019). R. verniciflua and E. ulmoides extract (ILF-RE) protects against chronic CCl_4-induced liver damage by enhancing antioxidation. Nutrients, 11(2), 38.

Lina, H. Z., Samy, M. M., Samir, A. B., Fatma, A. M., Kawther, M. T., & Abdelaaty, A. S. (2017). Hypoglycemic and antioxidant effects of *Hibiscus rosa-sinensis L*. leaves extract on liver and kidney damage in streptozotocin induced diabetic rats. African Journal of Pharmacy and Pharmacology, 11(13), 161-169.

Mughal, T.A., Ali, S., Hassan, A., Saleem, M.Z., Mumtaz, S., Mumtaz, S. (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), 1.

Mughal, T.A., Saleem, M.Z., Ali, S., Anwar, K.K., Bashir, M.M., Babar, M., Khan. M.A. (2019). Evaluation of Hepatotoxicity of Carbon Tetrachloride and Pharmacological Intervention by Vitamin E in Balb C mice. Pakistan Journal of Zoology. 51(2):755-61.

Mughal, T.A., Ali, S., Hassan, A., Kazmi, S.A.R., Saleem, M.Z., Shakir, H.A., Nazer, S., Farooq, M.A., Awan, M.Z., Khan, M.A. and Andleeb, S., (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., Ali, S., Tahir, H.M., Mumtaz, S., Mumtaz, S., Hassan, A. and Kazmi, S.A.R., (2020b). Anti-hyperlipidemic effect of Berberis lyceum Royle root bark extract in Alloxanized Swiss Albino mice. Punjab University Journal of Zoology, 35(2), 261-268.

Mughal, T. A., Ali, S., Khatoon, S., Khalil, S., & Mumtaz, S. (2023). Protective effect of Helianthus annuus seeds extract against CCl4-induced hepatocellular damage. Pakistan Journal of Biochemistry and Biotechnology, 4(2), 37-45.

Mughal, T. A., Ali, S., Nazar, S., Khatoon, S., & Khalil, S. (2024a). Hepato-protective potential of aqueous extract of Berberis lycium Royle root bark extract in alloxan induced diabetic Swiss albino mice. International Journal of Forest Sciences, 4, 47-58.

Mughal, T. A., Ali, S., Mumtaz, S., Summer, M., Saleem, M. Z., Hassan, A., & Hameed, M. U. (2024b). Evaluating the biological (antidiabetic) potential of TEM, FTIR, XRD, and UV-spectra observed berberis lyceum conjugated silver nanoparticles. Microscopy Research and Technique.

Nauroze, T., Ali, S., Kanwal, L., Ara, C., Mughal, T.A. and Andleeb, S., (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., Ali, S., Kanwal, L., Mughal, T.A., Andleeb, S. and Ara, C., (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.

Nazir, N., Muhammad, J., Ghaffar, R., Nisar, M., Zahoor, M., Uddin, F. ... & Alotaibi, A. (2021). Phytochemical profiling and antioxidant potential of Daphne mucronata Royle and action against paracetamol-induced hepatotoxicity and nephrotoxicity in rabbits. Nisar Saudi journal of biological sciences, 28(9), 5290-5301.

Okaiyeto, K., Nwodo, U., Mabinya, L., & Okoh, A. (2018). A review on some medicinal plants with hepatoprotective effects. Pharmacognosy Reviews, 12(24), 186-199.

Patel, S. (2015). Plant genus *Elaeagnus*: underutilized lycopene and linoleic acid reserve with permaculture potential. Fruits, 70(4), 191-199.

Perveen, R., Suleria, H. A. R., Anjum, F. M., Butt, M. S., Pasha, I., & Ahmad, S. (2015). Tomato (Solanum lycopersicum) carotenoids and lycopenes chemistry; metabolism, absorption, nutrition, and allied health claims—A comprehensive review. Critical reviews in food science and nutrition, 55(7), 919-929.

Rane, J., Jadhao, R., & Bakal, R. L. (2016). Liver diseases and herbal drugs:-A review. Journal of Innovations in Pharmaceutical and Biological Sciences, 3(2), 24-36.

Ritesh, K. R., Suganya, A., Dileepkumar, H. V., Rajashekar, Y., & Shivanandappa, T. J. T. R. (2015). A single acute hepatotoxic dose of CCl_4 causes oxidative stress in the rat brain. Toxicology reports, 2, 891-895.

Sharma, A. (2015). Monosodium glutamate-induced oxidative kidney damage and possible mechanisms: a mini-review. Journal of biomedical science, 22(1), 1-6.

Singh, D., Cho, W. C., & Upadhyay, G. (2016). Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. Frontiers in physiology, 6, 363.

Van der Paal, J., Neyts, E. C., Verlackt, C. C., & Bogaerts, A. (2016). Effect of lipid peroxidation on membrane permeability of cancer and normal cells subjected to oxidative stress. Chemical science, 7(1), 489-498.

Weber, L. W., Boll, M., & Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical reviews in toxicology, 33(2), 105-136.

Wu, M. C., Hu, H. T., Yang, L., & Yang, L. (2011). Proteomic analysis of up-accumulated proteins associated with fruit quality during autumn olive (*Elaeagnus umbellata)* fruit ripening. Journal of agricultural and food chemistry, 59(2), 577-583.

Xu, P., Yao, J., Ji, J., Shi, H., Jiao, Y., Hao, S. ... & Shi, H. (2019). Deficiency of apoptosis-stimulating protein 2 of p53 protects mice from acute hepatic injury induced by CCl_4 via autophagy. Toxicology Letters, 316, 85-93.

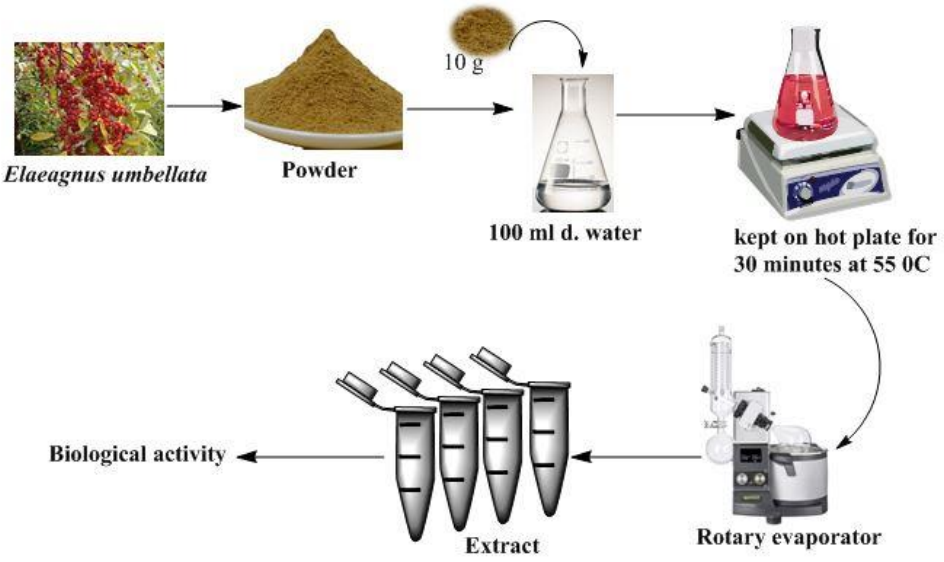

Figure 1: Preparation of Extract.

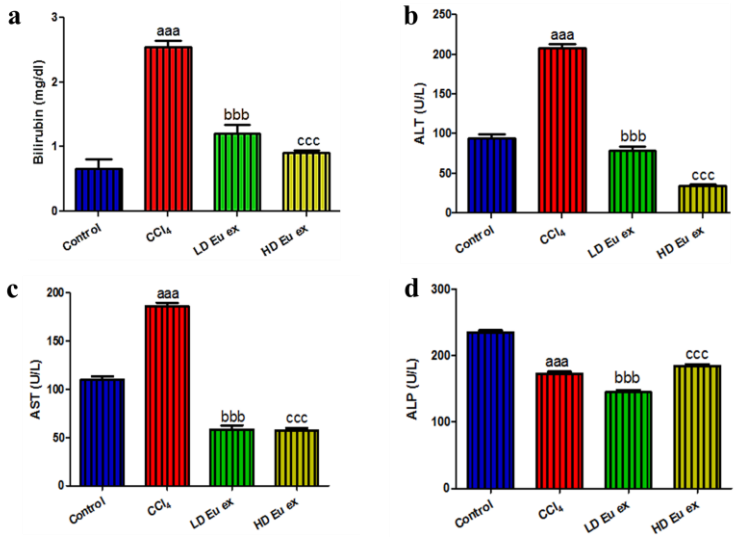

Figure 2: Effect on a. Bilirubin; b. ALT; c. AST; d. ALP; after exposure to *E. umbellata*, aaa, bbb, ccc = p≤ 0.001. (CCl4: Carbon tetrachloride; LD EU ex: Low dose of *E. umbellata* extract; HD EU ex: High dose of *E. umbellata* extract).

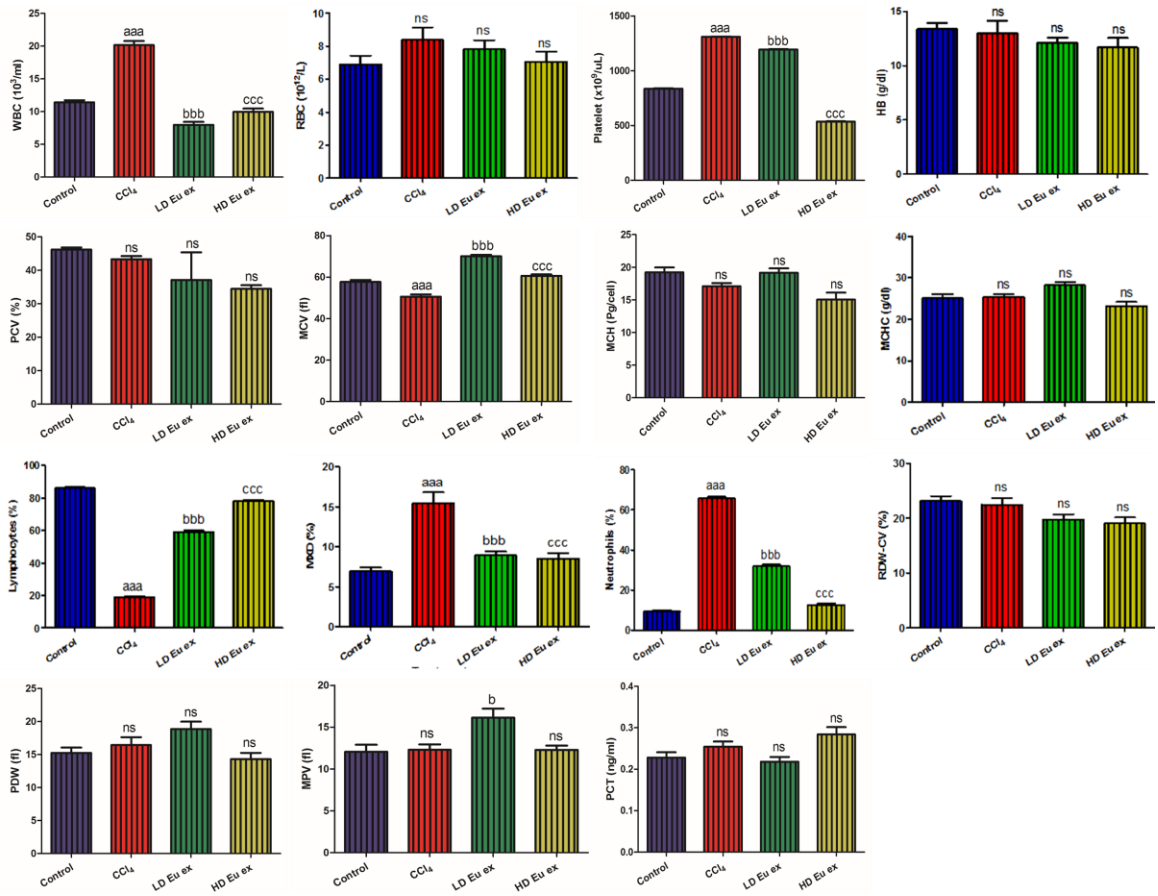

Figure 3: Effect on haematological parameters after exposure to *E. umbellata:* aaa, bbb, ccc = p≤ 0.001; b = p≤ 0.05; ns = non-significant. (CCl4: Carbon tetrachloride; LD EU ex: Low dose of *E. umbellata* extract; HD EU ex: High dose of *E. umbellata* extract).

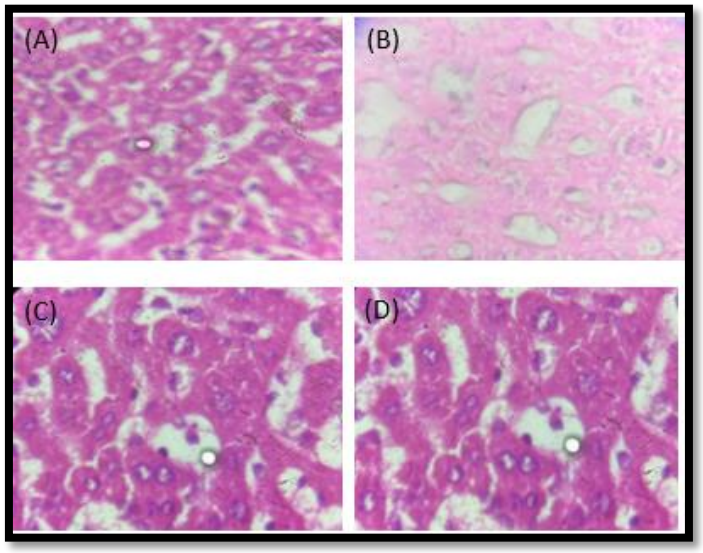

Figure 3: Effect on liver histology after exposure to *E. umbellata. A. Control; B.* CCl4 treated group; C. Low dose treated group of *E. umbellata* extract; D. High dose treated group of *E. umbellata* extract.