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Evaluation of Hepatoprotective Effect of *Rumex crispus* on CCl4-Induced Damage in Mice

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SUMMARY

Hepatotoxicity is the medical term for liver damage caused by a drug, chemical, herb, nutritional supplement, or exposure to xenobiotics such as food additives, alcohol, sulfur-containing solvents, peroxidized fatty acids, and fungal toxins. Several medicinal plants have been investigated for their potential hepatoprotective activity. The plant *Rumex crispus* is used as a folk medicine in Azad Kashmir, Pakistan. The purpose of the current study was to evaluate the hepatoprotective effect of leaf extract from *Rumex crispus* in Swiss Albino mice. Liver injury was induced by carbon tetrachloride (CCl4) in mice, and then these mice were treated *with R. Crispus* extracts. Hematology, histology, and liver biomarker tests were performed. The results showed that aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin levels in the serum of post-treated mice significantly increased compared to the negative control. Histopathological evidence supported these findings. The findings suggest that the *Rumex crispus* plant may possess hepatoprotective properties that are mainly mediated by this plant.

Keywords: Phytochemical, *Rumex crispus*, Liver damage

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INTRODUCTION

Liver can generate helpful principles and effectively detoxify xenobiotics. Thus, the consequences of liver damage or fibrosis caused by hepatotoxic substances are severe (Shan et al., 2019). Oxidative stress, inflammation, and liver cell death are some of the cellular and molecular pathways described to explain fibrosis and its prevention (Kisseleva and Brenner 2021). The main cause of the onset and advancement of fibrosis is chronic liver inflammation, which is also linked to the activation of macrophages in many liver disorders (Calvente et al., 2019). Without proper treatment, hepatic fibrosis, a major side effect of liver damage, could progress to liver cirrhosis. The most efficient treatment is to stop its progression (Friedman and Pinzani, 2022).

To reduce side effects and drug-drug interactions, it is essential to find a natural preventative agent that can interfere with fibrogenesis. In addition to the high death rate globally, fibrogenesis also causes a financial strain on all communities. Despite the emergence of several reports detailing interactions, natural flavonoids

derived from plant sources are highly regarded treatments for liver damage and fibrosis (Pan et al., 2020). Botanicals are a significant source of chemicals with potential for medical use. Botanical medicines, which are derived from plants, are significant contributors to the healthcare sector (Chugh et al., 2018).

The perennial plant *Rumex crispus* is a member of the Polygonaceae family. Because of its potent therapeutic properties, *R. crispus* has a long history of home herbal use. According to reports, the crude extract has qualities that are anti-inflammatory, antiseptic, antioxidant, and anti-diabetic (Feduraev et al., 2019; Minh et al., 2019; Eom et al., 2020).

MATERIALS AND METHODS

Research work was performed in Microbiology, Biotechnology and Toxicology Laboratory and Animal house of Women University of AJK Bagh.

COLLECTION OF SAMPLE

Rumex crispus was collected from local area of Rawalakot Ajk. The leaves of *Rumex crispus* dried under shade at room temperature. The dried leaves were grinded into fine powder.

PREPARATION OF EXTRACT

Fine powder used for the preparation of an aqueous extract according to a standard maceration protocol. Approximately 10 grams of powder were added to 200 ml of methanol and left for extraction and evaporation for 2 weeks. The dried extract was collected for dose preparation (Figure 1).

EXPERIMENTAL ANIMALS

Male Swiss Albino mice were obtained from the National Institute of Health (NIH) in Islambad, Pakistan. The mice were 4-5 weeks old and weighed approximately 30-35 g at the beginning of the experiment. They were kept in a controlled environment with a temperature of 22-25 oC, a 12-hour light/12-hour dark cycle, and had ad libitum access to food and water. The experiment was conducted during the light phase of the cycle. The animals were acclimated in the laboratory for a minimum of 24 hours before testing and were used only once.

ANIMAL GROUPING

Group I - control group, where these animals did not receive any sort of treatment. Group II - CCl4-induced diseased group. Group III - CCl4-induced low dose (200 mg/kg) treated group with Rc extract. Group IV - CCl4-induced high dose (400 mg/kg). The animals were treated with oral daily doses of Rc extract for 14 consecutive days.

HEPATOPROTECTIVE ACTIVITY

Swiss albino mice were subjected to CCl4 to induce hepatocellular damage. The liver of male Swiss albino mice was damaged by CCl4, and thereafter they were treated based on their respective groups. At the conclusion of the experiment, the mice were sacrificed and blood and liver tissues were collected for further analysis.

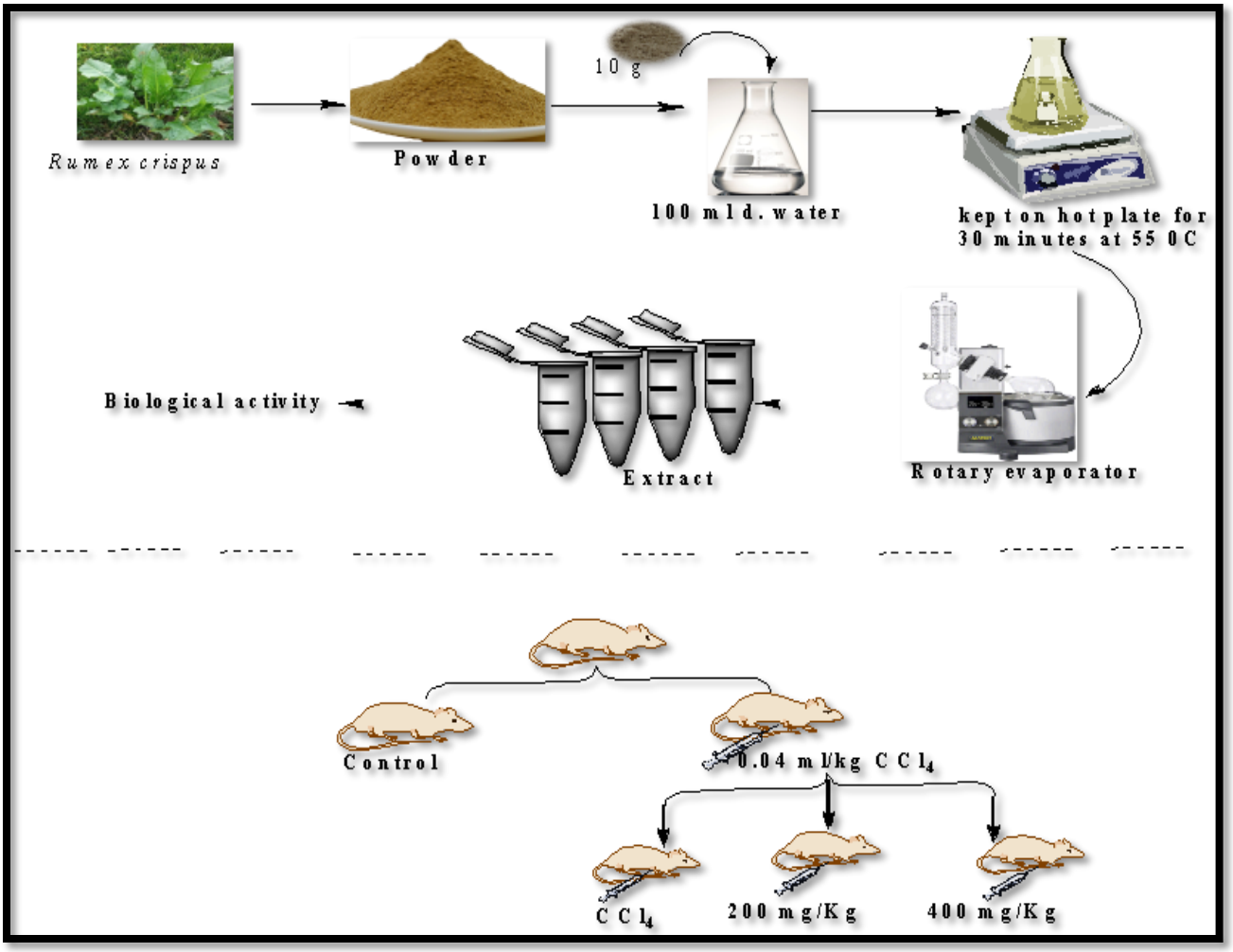

Figure 1: Schematic protocol for of extract preparation and hepatoprotective activity

LIVER FUNCTION TEST

Liver biomarkers tests Alanine transaminase (ALT), Aspartate transaminase (ATS), Alkaline Phosphate (ALP), were performed.

HISTOPATHOLOGICAL STUDIES

Livers of all mice were collected and histology was performed. Hematoxylin and Eosine stained specimens were observed under microscope.

STATISTICAL ANALYSIS

Data was analyzed by Graph pad Prism (version 5). All values were expressed as mean standard error of the mean (SEM). A one-way ANOVA with Bonferroni test was used to assess arithmetical variance among various groups. At p 0.05, values were considered to be statistically significant.

RESULTS

EFFECT ON LIVER FUNCTION TESTS

The current study evaluated the effectiveness of *Rumex crispus* leaf extract as a hepatoprotective agent. The levels of bilirubin, ALT, AST, and ALP significantly recovered in the RC LD (*Rumex crispus* extract low dose) and RC HD (*Rumex crispus* extract high dose) groups. The levels of bilirubin (2.54±0.10), ALT (212±0.70), AST (191.2±1.62), and ALP (330.4±27.1) significantly increased in the CCL4-induced disease group compared to the control normal group (bilirubin: 0.66±0.15, ALT: 94.2±1.39, AST: 110±3.53, ALP: 236±4.30). There was no significant recovery in the levels of bilirubin, ALT, AST, and ALP in the RC LD treated group. However, a significant decline was found in the levels of bilirubin (0.91±0.03), ALT (55.8±1.15), AST (105.8±1.62), and ALP (262.6±15.1) in the RC HD extract treated group, indicating hepatoprotective activity as shown in Figure 2.

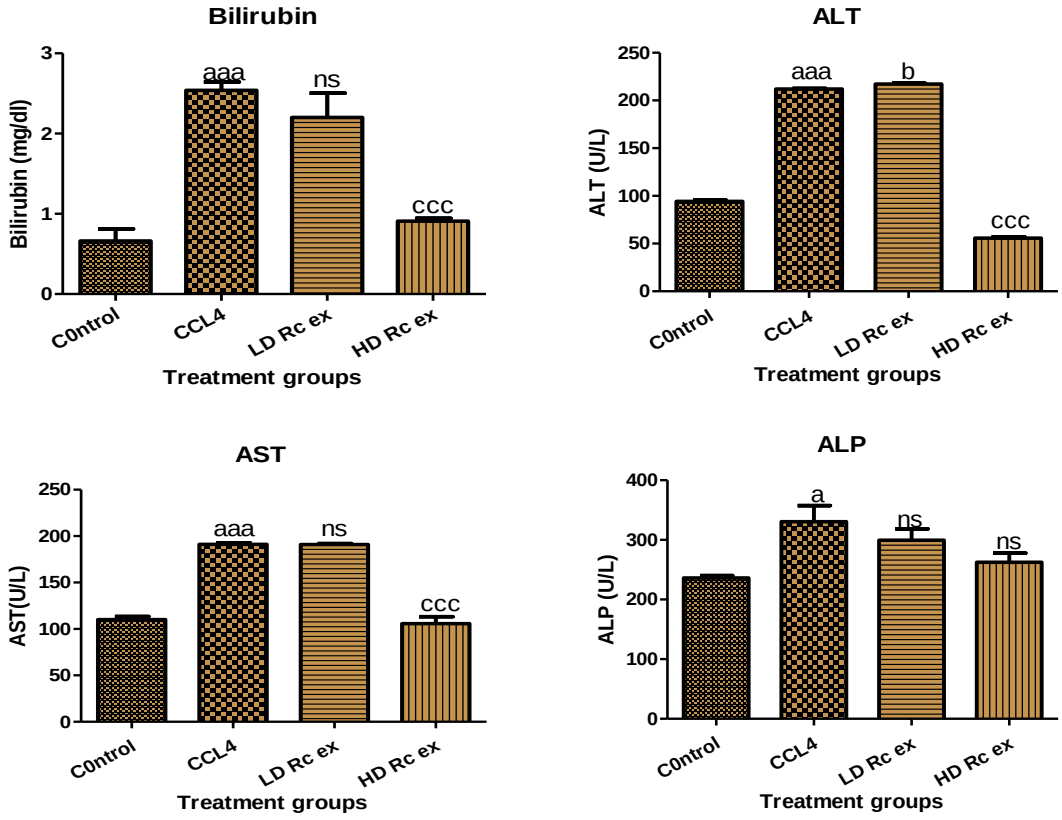

Figure 2. Enzymatic activity analysed between control normal group, CCL4-induced diseased group, RC LD treated group and RC HD treated group.

Key: [a]depicts significant difference between control and CCl_4. .[b] depicts significant difference between CCl_4 and low dose of Rc extract. [c]depicts significant difference between CCl_4 and high dose of Rc extract. Each bar represents mean value of five replicates and SEM. Statistical icon: aaa, ccc=≤ 0.001, b =≤ 0.05, a, =≤ 0.05.

HISTOPATHOLOGICAL ANALYSIS

Histopathological studies showed that ruptured cells were found in the CCL4-induced diseased and RC LD treated groups, as compared to the control normal group. However, less damage was observed in the RC HD treated group, indicating that the extract prevented liver cell damage in response to CCL4 induction (3).

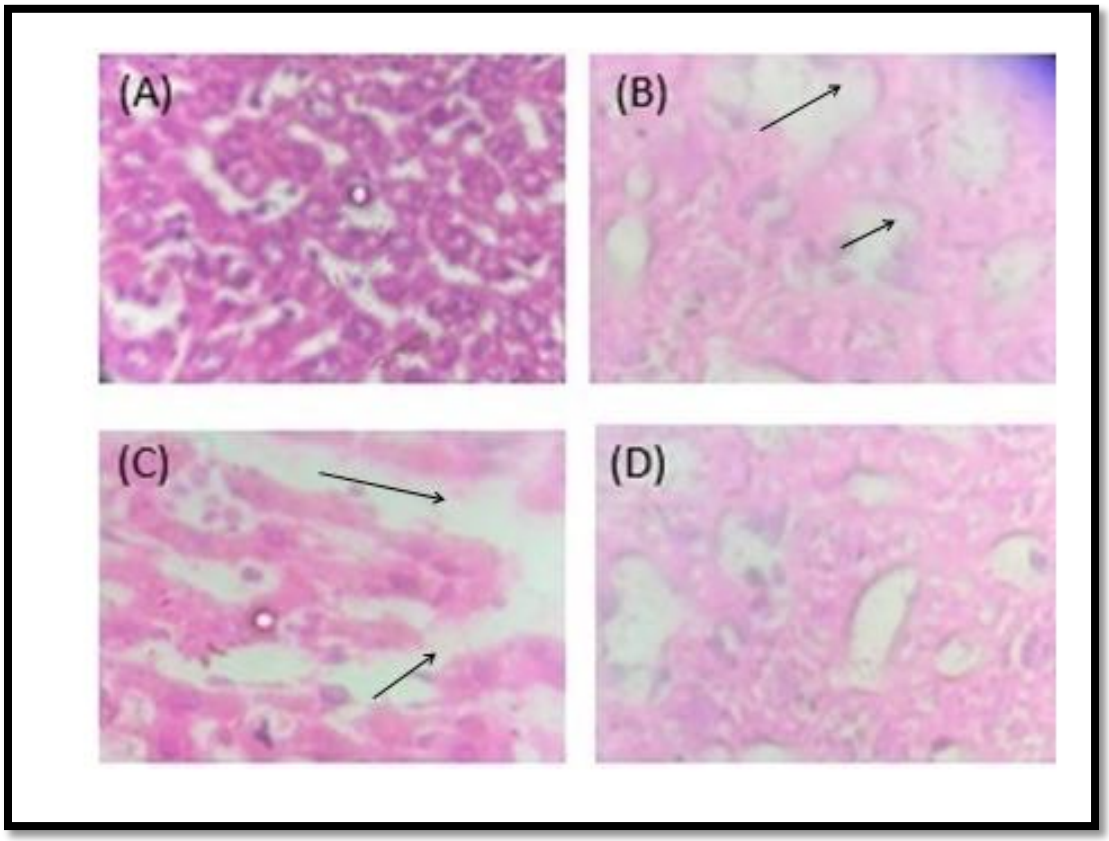

Figure 3: Histopathological photographs of livercells with H& E staining. A (control normal group), B (RC LD treated), C (CCL_4- induced diseased group) and D (RC HD treated group).

DISCUSSION

One of the xenobiotics that has been shown to cause acute and long-term liver tissue damage is a known hepatotoxin, carbon tetrachloride (CCl4) (Xu et al., 2010). CCl4 elevates serum indicators for nephrotoxicity and hepatotoxicity. Free radicals are crucial to the process of CCl4-induced liver and kidney damage (Khan et al., 2012). In the current study, the level of bilirubin, ALT, AST, and ALP were highly increased in the CCl4-induced diseased mice group, whereas a significant decline of bilirubin, ALT, AST, and ALP were found in the RC HD treated group when compared to the CCl4-induced diseased mice group. In addition to liver pathogenesis, it has been extensively utilized to research hepatotoxicity in animal models by starting lipid peroxidation and causing harm to the kidney, heart, testis, and brain (Khan et al., 2012). Earlier research demonstrated that by reducing lipid peroxidation and

increasing antioxidant enzyme activity, plant extracts high in phenolic compounds provided efficient defense against CCl4 hepatotoxicity (Huang et al., 2010).
Phytochemicals, which naturally develop in plants during metabolic processes and have a variety of defensive effects or disease-preventive capabilities, are non-nutritive chemical substances. It is known that plants create these compounds to safeguard themselves. Naturally occurring secondary metabolites that contain defensive mechanisms that shield against numerous diseases can be found in leaves, vegetables, and roots. Phytochemicals appear to neutralize free radicals, inhibit carcinogen-activating enzymes, and stimulate enzymes that detoxify carcinogens (Saxena, 2013; Minakshi et al., 2016).

Based on the current study, it was observed that the levels of all liver biomarkers were elevated in the diseased group, indicating hepatotoxicity. On the other hand, RC HD displayed a significant decline in liver function tests (LFTs), suggesting a protective effect of the extract against CCL4. These findings are consistent with previous studies (Adamu et al., 2020).

CONCLUSION

A certain amount of CCl4 in mice was found to be potentially harmful to the liver. The damage caused by CCl4 can be stopped by *Rumex crispus* extract. *R. crispus* was used for hepatoprotective activity. The present findings not only confirm the effectiveness of *R. crispus* but also imply its efficient hepatoprotective properties.

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REFRENCES

Adamu, B. A., B. Kefyalew, T. Tadele, B. Tilahun, A. Teklu and Z. Mekonnen. 2020. The role of community engagement on compassionate, respectful and caring health service: perceived effectiveness among health staffs in Ethiopia.

Calvente, C. J., M. Tameda, C.D. Johnson, H. Del Pilar, Y.C. Lin, N. Adronikou, and A.E. Feldstein. 2019. Neutrophils contribute to spontaneous resolution of liver inflammation and fibrosis via microRNA-223. The Journal of clinical investigation, 129(10): 4091-4109.

Chugh, N. A., S. Bali, and A. Koul. 2018. Integration of botanicals in contemporary medicine: road blocks, checkpoints and go-ahead signals. Integrative medicine research, 7(2): 109-125.

Eom, T., E. Kim and J.S. Kim 2020. In vitro antioxidant, antiinflammation, and anticancer activities and anthraquinone content from *Rumex crispus* root extract and fractions. Antioxidants, 9(8): 726.

Feduraev, P., G. Chupakhina, P. Maslennikov, N. Tacenko and L. Skrypnik. 2019. Variation in phenolic compounds content and antioxidant activity of different plant organs from *Rumex crispus L. and Rumex obtusifolius L*. at different growth stages. Antioxidants, 8(7): 237.

Friedman, S. L. and M. Pinzani. 2022. Hepatic fibrosis 2022: Unmet needs and a blueprint for the future. Hepatology, 75(2):473-488.

Huang, B., X. Ban, J. He, J. Tong, J. Tian, J. and Y. Wang. 2010. Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (*Nelumbo nucifera* Gaertn.) leaves. Food chemistry, 120(3): 873-878.

Khan, R. A., M. Khan, S. Sahreen. 2012. CCl_4-induced hepatotoxicity: protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement Altern Med. 8: 172-178.

Kisseleva, T. and D. Brenner. 2021.Molecular and cellular mechanisms of liver fibrosis and its regression. Nature Reviews Gastroenterology and Hepatology, 18(3): 151-166.

Minakshi, B., D. Jharna, E. Chanbi Devi, T. Nayan, P. Partha, N. Kundal and P.S. Manash. 2016. Phytochemical analysis of traditional medicinal plants and their antimicrobial activity: An experience from North East India. Open Access Journal of Pharmaceutical Research, 1(1): 000104.

Minh, T. N., T.M. Van, Y. Andriana, L. Vinh, D. Hau, D. Duyen and C. Guzman-Gelani. 2019. Antioxidant, xanthine oxidase, α-amylase and α-glucosidase inhibitory activities of bioactive compounds from *Rumex crispus L*. root. Molecules, 24(21):3899.

Pan, X., X. Ma, Y. Jiang, J. Wen, L. Yang, D. Chen and C. Peng. 2020. A comprehensive review of natural products against liver fibrosis: flavonoids, quinones, lignans, phenols, and acids. Evidence-Based Complementary and Alternative Medicine, 2020.

Saxena, M., J. Saxena, R. Nema, D. Singh and A. Gupta. 2013. Phytochemistry of medicinal plants. Journal of pharmacognosy and phytochemistry, 1(6).

Shan, L., Z. Liu, L. Ci, C. Shuai, C. Lv. and J. Li. 2019. Research progress on the anti-hepatic fibrosis action and mechanism of natural products. International Immunopharmacology, 75: 105765.

Xu, J.Y., Y. Su, J. Cheng, S. Li, R. Liu, and W. Li. 2010. Protective effects of fullerenol on carbon tetrachloride-induced acute hepatotoxicity and nephrotoxicity in rats. Carbon, 1388-1396.

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