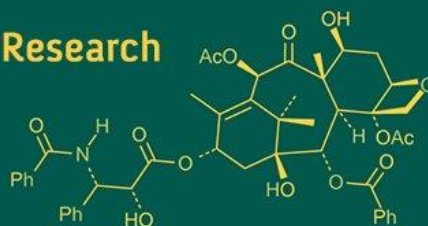
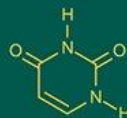
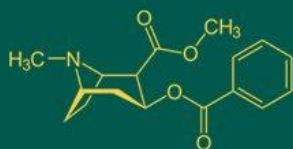


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(12): 350-356
www.biochemjournal.com
Received: 21-09-2025
Accepted: 25-10-2025

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Protective effects of garlic-derived lectin on carbon tetrachloride-induced hepatotoxicity in male wistar rats: Impacts on organ weight, hematological and biochemical parameters

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i12e.6508>

Abstract

This study aimed to assess the anti-inflammatory and antioxidant efficacy of a garlic-derived lectin in a rat model of carbon tetrachloride (CCl₄)-induced hepatotoxicity. Thirty-six age-matched rats were randomly assigned to six groups (n = 6): (I) normal control; (II) disease control (CCl₄ only); (III) positive control receiving silymarin (200 mg/kg, orally); (IV) garlic lectin 10 mg/kg; (V) garlic lectin 20 mg/kg; and (VI) garlic lectin 40 mg/kg. Liver injury was induced by intraperitoneal administration of CCl₄ (2 mL/kg in 50% vegetable oil) twice weekly for five weeks. From 6th to 8th week, rats were treated with silymarin (orally) or lectin (intraperitoneally) at the designated doses. Compared to the disease control group, lectin-treated animals particularly those receiving 20 mg/kg and 40 mg/kg showed significant improvement absolute and relative liver weight, suggesting hepatoprotection. Hematological assessments indicated restoration of red blood cell (RBC) counts, hemoglobin concentration, and packed cell volume (PCV) toward normal levels. The elevated total white blood cell (WBC) counts observed in CCl₄-exposed rats.

Biochemical analyses revealed marked normalization of liver function parameters: serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN, Creatinine, and alkaline phosphatase (ALP) levels were significantly lower in the lectin-treated groups versus disease control.

In conclusion, garlic-derived lectin administered at 20 mg/kg and 40 mg/kg demonstrates potent hepatoprotective and anti-inflammatory in rats with CCl₄-induced liver injury, as evidenced by changes in hematological and sero-biochemical parameters.

Keywords: Garlic lectin, Rat model, Hepatoprotection, Hematological parameters, Liver enzymes

Introduction

The liver is very vulnerable to toxic, infectious, endotoxin and oxidant related damage because of its major function in metabolism, detoxifying activities, Kupffer cell population and sentinel position between the splanchnic and systemic circulatory systems. Receiving 75 percent of its blood flow directly from the GI tract, acute and chronic enteritis can contribute to liver injury. Pancreatic inflammation imposes risks for obstructive cholestasis and hepatobiliary inflammation. Certain medications (NSAIDs, phenobarbitone, primidone, diazepam), bacteria, entero and endotoxins, toxins produced from moulds like aflatoxins, fungus, algae, spoiled or contaminated food, and transition metals such as arsenic and lead are only a few of the toxins that have been found to specifically cause liver injury (Jaeschke *et al.*, 2002)^[1].

Garlic has been used in traditional medicine as a food component to prevent the development of cancer and cardiovascular diseases, by modifying the risk factors such as hypertension, high blood cholesterol and thrombosis and preventing other chronic diseases associated with ageing (Rahman, 2001)^[2]. The presence of diallyl sulphide, diallyl disulfide, allicin and dipropyl sulphide, among other pharmacologically active sulphur compounds, is thought to be responsible for these pharmacological properties of garlic. These substances have been shown to have anti-oxidative properties, anti-inflammatory effects in vitro and in vivo, and to boost the activity of enzymes involved in the metabolism of carcinogens (Fisher *et al.*, 2007)^[3].

Materials and Methods

The present work “Studies on effects of Garlic lectin in Carbon tetrachloride induced hepatotoxicity in male wistar rats” was carried out during academic year 2021-22 at the department of Veterinary Pathology, Veterinary College, Hassan, KVAFSU, Bidar. The study was conducted on adult healthy male Wistar albino rats. Healthy adult male Wistar rats weighing 180-200 g. To induce experimental hepatotoxicity in rats, CCl₄ was procured from Sigma Aldrich Corporation, St. Louis, USA with molecular weight 153.82; CAS number 56-23-5. All other chemicals and reagents used for the study were of analytical grade. It was injected intraperitoneally at the rate of 2ml/kg body weight 50 percent in vegetable oil twice a week for five weeks (Elsawy *et al.*, 2019) [21]. Garlic derived lectin extracted purified and characterized was procured from Department of Biochemistry, Davangere University, Shivangotri, Davangere. The working injectable Garlic lectin solution was prepared in fresh phosphate buffered saline having a pH of 7.4 and the same was maintained at 4-8 °C. The required quantity of garlic lectin was dissolved in ice-cold phosphate buffered saline and injected intraperitoneally to rats immediately to avoid degradation. Silymarin available commercially as oral hepatoprotective tablets (silybon® - 70) 70 mg tablet manufactured by Micro lab ltd), was purchased and administered orally at the dose rate of 200mg/kg bw / weekly four times for three weeks from six to eight weeks of study (Tsai *et al.*, 2008) [4].

Experimental protocol

Rats were selected randomly and divided into six groups (Group I to VI) each consisting of six rats. Group I served as normal control group and Group II served as CCl₄ disease control. All the rats were numbered group wise and individually. Hepatotoxicity was induced in Group II to VI animals using Carbon tetrachloride (50 % in Vegetable oil) @ 2 ml/kg bw twice a week for first five weeks. The effects of CCl₄ treatment and induction of hepatotoxicity was studied in the rats of Group II that served as CCl₄ disease control.

Parameters assessed

Organ weight Liver, Relative liver weight, Hematological Parameters (TEC, TLC, HB, Platelet), Biochemical Parameters (AST, ALT, ALP, Creatinine, Total Protein, BUN).

Results

Absolute Organ weight

The mean values and percentage difference of absolute liver weight in g with standard error of mean on 56th day of the experiment have been presented in Table 1.

The mean values of absolute liver weight with standard error of mean of Group I (Normal control) and Group II (Disease control) were 7.95±0.32 and 10.54±0.07, respectively. There was a significant ($p<0.05$) increase in liver weight of disease control in comparison to normal control group (Group I).

The mean values of absolute liver weight with standard error of mean of Group III, IV, V and VI were 8.19±0.18, 8.49±0.19, 8.3±0.33 and 8.25±0.25, respectively. The mean values of Group III to VI were decreased in comparison with Group II. There was a significant ($p<0.05$) decrease in liver weight of Group III, IV, V and VI rats in comparison

to Group II rats and also not significant when compared to Group I.

Relative Liver Weight

The mean values of relative liver weight was calculated by taking of percentage ratio of absolute liver weight and body weight in grams with standard error of mean of Group I (Normal control) and Group II (Disease control) were 3.01±0.11 and 3.88±0.13, respectively. There was a significant ($p<0.05$) increase in liver weight of disease control in comparison to normal control group (Group I).

The mean values of relative liver weight with standard error of mean of Group III, IV, V and VI were 3.43±0.11, 3.65±0.12, 3.19±0.11 and 3.22±0.12, respectively. The mean values of Group III to VI were decreased in comparison with Group II. There was a significant ($p<0.05$) decrease in liver weight of Group III, IV, V and VI rats in comparison to Group II rats also Group IV rats were comparable with that of Group II rats and Group III when compared Group I difference in the value were statistically significant ($P<0.05$) in Group IV further the values of Group V and VI were comparable with that of Group I rats.

Haematology Parameters

The mean values and percentage difference of Haemoglobin (Hb) in g/dL with standard error of mean on 56th day of the experiment have been presented in Table 2.

Haemoglobin (Hb)

The mean values of Hb (g/dl) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 15.1±0.63 and 12.36±0.17, respectively. There was a significant ($p<0.05$) decrease in Hb of Disease control (18.1 %) in comparison to normal control group.

The mean values of Hb with standard error of mean of Group III, IV, V and VI were 14.2±0.36, 14.48±0.24, 14.08±0.33 and 14.44±0.28 respectively. The mean values of Hb (g/dl) of Group III to VI were increased in comparison with Group II.

The percentage increase in Hb value of Group III, IV, V and VI in comparison to Group II were 14.9, 17.2, 13.9, and 16.8 percent, respectively.

The percentage decrease in Hb value of Group III, IV, V and VI in comparison to Group I rats were 6.0, 4.1, 6.8 and 4.4 percent, respectively. However, these values were statistically not significant.

Total erythrocyte count (TEC)

The mean values of TEC (106/μl) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 5.48±0.21 and 4.57±0.12, respectively. There was a significant ($p<0.05$) decrease in TEC of disease control (15.4 %) in comparison to normal control group.

The mean values of TEC (106/μl) with standard error of mean of Group III, IV, V and VI were 5.22±0.23, 5.3±0.15, 5.15±0.15 and 5.4±0.09, respectively. The mean values of TEC of Group III to VI were increased significantly ($p<0.05$) in comparison with Group II rats. The percentage increase in TEC of Group III, IV, V and VI in comparison to Group II were 14.2 per cent, 16.0, 12.9, and 18.2 percent, respectively.

The percentage decrease in TEC value of Group III, IV and V in comparison to Group I rats were 3.3, 1.9 and 4.4

percent, respectively. The values of Group III to VI were statistically not significant with that of Group I rats.

Total Leucocyte count (TLC)

The mean values of TLC (103/ μ l) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 12.16 ± 1.35 and 16.17 ± 0.2 , respectively. There was a significant ($p < 0.05$) increase in TLC of disease control (33.0 %) in comparison to normal control group.

The mean values of TLC (103/ μ l) with standard error of mean of Group III, IV, V and VI were 12.84 ± 2.02 , 13.01 ± 1.13 , 12.72 ± 0.29 and 12.38 ± 0.35 , respectively. There was a significant ($p < 0.05$) decrease in TLC of Group V and VI in comparison to Group II rats. The percentage decrease in TLC value of Group III, IV, V and VI in comparison to Group II were 20.5, 19.6, 21.3, and 23.4 percent, respectively.

The percentage increase in TLC value of Group III and IV in comparison to Group I rats were 5.7 and, 6.9 percent, respectively. The Group V and VI rats showed 4.6 and 1.8 percent decrease, respectively and the values were comparable with that of Group I rats.

Packed Cell Volume (PCV)

The mean values of PCV (%) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 45.3 ± 1.88 and 37.08 ± 0.52 , respectively. There was a significant ($p < 0.05$) decrease in PCV of disease control (18.1 %) in comparison to normal control group.

The mean values of PCV (%) with standard error of mean of Group III, IV, V and VI were 42.6 ± 1.07 , 43.44 ± 0.72 , 42.24 ± 0.98 and 43.32 ± 0.83 respectively. There was a significant ($p < 0.05$) increase in PCV of Group III to VI in comparison to Group II. The percentage increase in PCV value of Group III, IV, V and VI in comparison to Group II were 14.9, 17.2, 13.9, and 16.8 percent, respectively.

The percentage decrease in PCV value of Group III, IV, V and VI in comparison to Group I rats were 6.0, 4.1, 6.8 and 4.4 per cent, respectively.

Platelet count

The mean values of Platelet (103/ μ l) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 431.1 ± 22.94 and 303.3 ± 17.8 , respectively. There was a significant ($p < 0.05$) decrease in Platelet of disease control (29.6 %) in comparison to normal control group.

The mean values of Platelet (103/ μ l) with standard error of mean of Group III, IV, V and VI were 410.01 ± 21.31 , 380.6 ± 15.04 , 390.8 ± 35.63 and 401.2 ± 49.23 , respectively. There was a significant ($p < 0.05$) increase in Platelet of Group III to VI in comparison to Group II and were comparable to Group I rats. The percentage increase in Platelet value of Group III, IV, V and VI in comparison to Group II were 35.17, 25.48, 28.84, and 32.27 percent, respectively.

The percentage decrease in Platelet value of Group III, IV, V and VI in comparison to Group I were -4.89, -11.71, -9.3 and -6.9 percent, respectively. The mean values were significantly ($p < 0.05$) higher when compared to Group I.

Serum Biochemistry Parameters

The mean values and percentage difference of serum aspartate aminotransferase (AST), Alanine Transaminase

(ALT) and Serum Alkaline phosphatase (ALP) in IU/L. Serum blood urea nitrogen (BUN) and Serum Creatinine in mg/dl. Serum Total Protein in g/dl with standard error of mean on 56th day of the experiment have been presented in Table 3.

Serum Aspartate Aminotransferase (AST)

The mean values of AST (IU/L) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 110.23 ± 3.42 and 189.77 ± 10.42 , respectively. There was a significant ($p < 0.05$) increase in AST (IU/L) of disease control (71.6 %) in comparison to normal control group.

The mean values of AST (IU/L) with standard error of mean of Group III, IV, V and VI were 153.9 ± 8.23 , 143.65 ± 11.38 , 146.4 ± 11.40 and 147.402 ± 6.69 , respectively. The mean AST (IU/L) values of Group III to VI were decreased in comparison to Group II. The percentage decrease in AST value of Group III, IV, V and VI in comparison to Group II were 18.7, 21.0, 24.1, and 22.6 percent, respectively and the difference in the values were statistically significant ($p < 0.05$). The percentage increase in AST value of Group III, IV, V and VI in comparison to Group I rats were 39.6, 35.5, 30.3 and 32.8 percent, respectively and difference in the value were statistically significant ($p < 0.05$).

Serum Alanine Transaminase (ALT)

The mean values of ALT (IU/L) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 73.1 ± 3.02 and 105.3 ± 3.31 respectively. There was a significant ($p < 0.05$) increase in serum ALT of disease control (44.5 %) in comparison to normal control group (Group I).

The mean values of ALT (IU/L) with standard error of mean of Group III, IV, V and VI were 82.12 ± 4.26 , 84.2 ± 4.34 , 80.80 ± 5.44 and 76.93 ± 3.704 , respectively. There was a significant ($p < 0.05$) decrease in serum ALT of Group III, IV, V and VI rats in comparison to Group II rats. The percentage decrease in ALT value of Group III, IV, V and VI were 22.2, 20.6, 23.2 and 27.5 percent, respectively in comparison to Group II rats. These values of Group III, IV, V and VI differed significantly ($p < 0.05$) lower when compared to Group I.

The percentage increase in ALT value of Group III, IV, V and VI compared to Group I were 12.3, 14.6, 11.0 and 4.8 percent, respectively. The values of Group III, IV, V and VI differed statistically ($p < 0.05$) in comparison to Group I.

Serum Alkaline phosphatase (ALP)

The mean values of ALP (IU/L) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 276.1 ± 16.4 and 501.83 ± 88.07 , respectively. There was a significant ($p < 0.05$) increase in serum ALP (IU/L) of disease control (81.5 %) in comparison to normal control group.

The mean values of ALP (IU/L) with standard error of mean of Group III, IV, V and VI were 385.16 ± 36.22 , 341.8 ± 28.42 , 331.93 ± 17.97 and 332 ± 27.37 , respectively. There was a significant ($p < 0.05$) decrease in serum ALT (IU/L) of Group IV, V and VI rats in comparison to Group II rats. The percentage decrease in ALP value of Group III, IV, V and VI compared to Group II were 23.2, 31.9, 33.9, and 33.8 percent, respectively.

The percentage increase in ALP value of Group III, IV, V and VI compared to Group I were 39.3, 23., 20.0 and 20.1

percent, respectively. These values however were statistically not significant.

Serum blood urea nitrogen (BUN)

The mean values of BUN (mg/dl) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 10.97 ± 0.81 and 18.42 ± 1.83 , respectively. There was a significant ($p < 0.05$) increase in serum BUN (mg/dl) of Disease control (67.9 %) in comparison to normal control group.

The mean values of BUN (mg/dl) with standard error of mean of Group III, IV, V and VI were 14.06 ± 1.48 , 15.37 ± 2.92 , 11.79 ± 0.68 and 12.44 ± 2.01 , respectively. There was a significant ($p < 0.05$) decrease in BUN (mg/dl) of Group III, V and VI rats in comparison to Group II rats. The percentage decrease in BUN value of Group III, IV, V and VI compared to Group II were -23.7, -16.8, -36.0, and -32.5 percent, respectively.

The percentage increase in BUN value of Group III, IV, V and VI compared to Group I were 28.2, 16.5, 7.5 and 13.4 percent, respectively. These values however were statistically not significant.

Serum Creatinine

The mean values of creatinine (mg/dl) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 0.45 ± 0.04 and 0.85 ± 0.05 , respectively. There was a significant ($p < 0.05$) increase in serum creatinine (mg/dl) of disease control (88.9 %) in comparison to normal control group.

The mean values of creatinine (mg/dl) with standard error of mean of Group III, IV, V and VI were 0.48 ± 0.04 , 0.59 ± 0.03 , 0.55 ± 0.04 and 0.51 ± 0.01 , respectively. There was a significant ($p < 0.05$) decrease in creatinine of Group III to VI rats in comparison to Group II rats. The percentage decrease in creatinine value of Group III, IV, V and VI compared to Group II were -43.5, -30.5, -66.7 and -40 percent, respectively. The percentage decrease in creatinine value of Group III, IV, V and VI compared to Group I were 6.7, 31.1, 22.2 and 13.3 percent, respectively. These values however were statistically not significant.

Serum Total Protein

The mean values of Total protein (g/dl) with standard error of mean of Group I (Normal control) and Group II (Disease control) were 5.58 ± 0.09 and 4.31 ± 0.39 , respectively. There was decrease in 22.8 percent in Group II when compared to normal control group. The values differed statistically ($p < 0.05$).

The mean values of total protein (g/dl) with standard error of mean of Group III, IV, V and VI were 5.08 ± 0.49 , 4.902 ± 0.08 , 5.20 ± 0.21 and 5.4 ± 0.19 , respectively. The percentage increase in Total protein value of Group III, IV, V and VI compared to Group II were 17.9, 13.7, 20.6 and 25.3 percent respectively. The mean values of Group III to VI were increased significantly compared to Group II. However in Group V and VI values were increased significantly ($p < 0.05$) compared to Group II and Group V is comparable with Group III.

The percentage decrease in total protein (g/dl) value of Group III, IV, V and VI compared to Group I were 9.7, 12.2, 6.8 and 3.2 percent, respectively. The values differed significantly with Group I.

Discussion

Liver Weight

In present study, administration of 50 percent carbon tetrachloride in vegetable oil @ 2 ml/kg bw twice a week intraperitoneally for five weeks in Group II disease control rats resulted in significant increase in absolute and relative liver weight in comparison to the relative liver weight of Group I normal control rats the reduction of body weights, the livers of the CCl₄ treated rats showed enlargement and increment in weight due to the infiltration of fatty acids and glycerols into the hepatocytes upon damage of the cell membranes induced by CCl₄ (Aouadi *et al.*, 2011) [5].

The mean absolute and relative liver weight of Group III rats were significantly lesser compared to that of disease control rats (Group II). The results indicated that silymarin treatment showed significantly normalized the increase in relative weight associated with CCl₄ induced hepatotoxicity. This could be attributed to anti-inflammatory and antioxidant effects of silymarin (Doughri *et al.*, 2014) [6]. The absolute liver weight was comparable with Group I. However, the mean relative liver weight of Group III rats was found to be significantly higher when compared with normal control rats.

The absolute liver weights of garlic lectin treatment Groups IV to VI were significantly lower when compared to Group II and comparable with Group I. The relative liver weight of treatment Groups V and VI rats were significantly lower ($P < 0.05$) when compared to disease control rats (Group II). However Group IV rats were comparable with disease control group. However the low dose of garlic lectin was comparable with disease control group. The relative liver weights of rats of Group V and VI was comparable with corresponding values of normal control group and also reference control group that suggested that middle dose and high dose of garlic lectin decreased the relative liver weight. Garlic supplementation might have inhibited the activity of enzymes related to liver adipogenesis and regulation of lipogenesis, decreased intestinal absorption of TGs, and would have improved dyslipidemia leading to decrease in liver weight (Ha *et al.*, 2015) [7]. Similarly, garlic lectin also would have acted leading to decrease in liver weight.

Haematology parameters

In the present study, all rats in the normal control group (Group I) remained healthy throughout the study period with normal hematological parameters (Gad, 2007) [8].

The study demonstrated that CCl₄ administration produced pancytopenia as shown by microcytic hypochromic anaemia, thrombocytopenia and lymphopenia in the blood as evidenced by the reduction in the PCV, RBC and platelets with the exception of total WBC counts this reduction in the formed elements in the blood is stress induced because of the leucocytosis (Swenson, 1993) [9]. The release of CCl₄ reactive species trichloromethyl and trichloromethyl peroxy caused the transient decrease in the Hb concentration and PCV level due to hemolytic anemia caused by oxidation of sulphhydryl groups of the erythrocyte membrane in addition to disturbing hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and/or their enhanced removal from circulation. Carbon tetrachloride can cause haemolytic anaemia when sulphhydryl groups of the erythrocyte membrane is oxidized which inflicts injury to the erythrocyte membrane (Fairbanks, 1967; Abu *et al.*, 2022) [10, 11].

In the present study, total erythrocyte count, haemoglobin, packed cell volume and platelet of Group III rats were significantly higher when compared to Group II disease control rats and comparable to that of normal control rats. Silymarin has anti-inflammatory effects through reduction of TNF- α , protective effects on erythrocyte lysis (Karimi *et al.*, 2011) [12]. The cytoprotective effects of Silymarin are mainly attributable to its antioxidant and free radical scavenging properties (Ali *et al.*, 2015) [13].

In the present study, the rats in treatment Group IV to VI rats showed significant increase in total erythrocyte count, haemoglobin, packed cell volume and platelet count when compared to disease control (Group II) rats and all the treatment groups were comparable with Group III and also with Group I. This may be attributed to the antioxidant property of garlic lectin and protection of cell membrane of the affected cells (Hanamantappa, 2022).

In the disease control group rats, there was a significant ($P<0.05$) increase in the mean total leucocyte count in comparison to Group I normal control. Similar findings were also noticed by earlier worker (El-Bialy *et al.*, 2019) [15]. The CCl₄ treatment significantly increased WBCs count which may be attributed to lymphocyte infiltration of poisoned cells, a clear case of immune response to a chemical antigen by the body defensive mechanism of immune system (Saba *et al.* 2010) [16]. Acute stress in animals including birds have been widely reported to be associated with increased white blood cell count occasioned by significant increase in the neutrophil count and the neutrophil/lymphocyte ratio (Larson *et al.*, 1985) [17].

The mean TLC levels of the rats in treatment Group III was lower compared to Group II and comparable to that of normal control rats. The results indicated that silymarin could bring back the TLC increased by CCl₄ to normal level. Similar observation was made by Robert *et al.* (2021) [18].

The mean TLC levels of the rats in treatment with garlic lectin in Group V and VI were decreased significantly when compared to Group II and were comparable with Group III and Group I. However, at low dose of garlic lectin (Group IV) there was decrease in leucocyte but it was comparable to Group II. Similar findings have been observed in members of other lectin family such as soybean agglutinin which is known to possess an inhibitory effect on neutrophil count and their migration, indicating an anti-inflammatory action.

Serum Biochemistry

In the present study, all rats in the normal control group (Group I) remained healthy throughout the study period with normal serum biochemistry parameters.

In the present study, there was significant decrease in AST, ALT level of Group III rats when compared to Group II. However, the decrease in ALP value was not significant when compared to Group II. Due to its phenolic nature, it is capable of donating electrons to stabilize free radicals and reactive oxygen species (ROS). Silymarin also affects intracellular glutathione, which prevents lipoperoxidation of membranes (Karimi *et al.*, 2011) [12] and also Silymarin extract inhibited the CCl₄-induced hepatic TNF- α , - α and IL-1 β expression.

Among the garlic lectin treatment groups, Group IV to VI showed significant decrease in AST, ALT, ALP value when compared to Group II rats and the treatment groups were comparable with reference control group. However, the

values were significantly higher when compared to Group I. This may be attributed to the antioxidant property of garlic lectin and protection of cell membrane of the affected cells (Hanamantappa, 2022). Garlic as been proposed to have anti-inflammatory and immunomodulatory effects, garlic metabolites have been implicated in reduction of nuclear factor (NF)- κ B activity in turn leading reduction of pro-inflammatory cytokines such as IL-1 β and TNF- α , in liposaccharide induced human inflammatory model and invitro system (Keiss *et al.*, 2003) [19].

In the disease control group rats, a significant ($P<0.05$) increase in the mean values of BUN and creatinine were observed. Similar observation was also made by several earlier researcher (Akram *et al.*, 2019, Elsayy *et al.*, 2019) [20, 21].

Carbon tetrachloride, besides exerting its toxic effect on the liver, also reportedly gets distributed at higher concentrations in the kidney than in the liver. The mechanism of CCl₄-renal toxicity is almost same as that of the liver, but CCl₄ shows a high affinity to the kidney cortex which contains cytochrome P-450 predominantly (Jaramillo-juarez *et al.*, 2008) [22].

In the present study, the mean value of BUN and creatinine level of Group III rats was decreased significantly when compared to Group II and also values were comparable with that of Group I. Silymarin has an antioxidant, free radical scavengers, intracellular glutathione GSH regulator and stabilizing cell membrane activity (Ahmed *et al.*, 2019) [23].

Among the garlic lectin treatment groups, Groups IV to VI showed significant increase in mean creatinine value when compared to Group II rats and also was comparable with Group III and I. There was significant increase in mean value of BUN of Group V and VI when compared to Group II and was also comparable with Group III and I. However, Group IV showed no significant difference with Group II and Group I. The beneficial effects of garlic lectin on kidneys might be due to decrease in ROS production and down regulation of apoptotic mechanisms induced by CCl₄. Similar findings have been reported in other families of plant derived lectins such as Fabaceae lectin Diocleaviolacea (Dvl) with proven beneficial effects in treating acute kidney damage. The mechanisms proposed for such protective effects include decrease in the renal vascular resistance and decrease in apoptosis and ROS production.

In the present study, there was a significant decrease in total protein of CCl₄ treated disease control rats in comparison to normal control Group rats. The decrease in total serum protein in CCl₄ treated rats might be due to the decrease in the number of functional hepatocytes which in turn, may result in decreased hepatic capacity to synthesize protein (Saba *et al.*, 2010) [16].

The mean value of total serum protein level of Group III rats when compared to Group II the values were increased significantly ($P<0.05$) and was also significantly lowered when compared to Group I. Due to its phenolic nature, it is capable of donating electrons to stabilize free radicals and reactive oxygen species (ROS). Silymarin also affects intracellular glutathione, which prevents lipoperoxidation of membranes (Karimi *et al.*, 2011) [12].

Among the garlic lectin treatment groups, Group IV to VI showed significant decrease in mean total serum protein value when compared to Group II rats. The Group VI values were increased significantly when compared with Group III and was comparable with normal control group. These

improvements in the treatment group could be due to the suppressive action on the mediators of inflammation like TNF- α , and IL-1b (Hanamantappa, 2022). TNF-alpha and IL-1 inflammatory cytokines stimulate the activity of

anorectic proopio melanocortin neurons and inhibit the activity of orexigenic neuropeptide Y (NPY) neurons leading to reduction of appetite in man and laboratory animals (Shinsyu *et al.*, 2020) [25].

Table 1: The mean (\pm SE) absolute and relative liver weight of different groups of rats

GROUPS	Absolute liver weight in g	Final day body weight	Relative liver weight with respect to body weight as percentage
Group I (NC)	7.95 \pm 0.32 ^a	269.67 \pm 10.65	3.01 \pm 0.11 ^a
Group II (DC)	10.54 \pm 0.07 ^b	225.5 \pm 7.16	3.88 \pm 0.13 ^d
Group III (RC)	8.19 \pm 0.18 ^a	257.5 \pm 7.07	3.43 \pm 0.11 ^{bc}
Group IV (GL 10)	8.49 \pm 0.19 ^a	245.33 \pm 18.9	3.65 \pm 0.12 ^{cd}
Group V (GL 20)	8.3 \pm 0.33 ^a	247.5 \pm 11.97	3.19 \pm 0.11 ^{ab}
Group VI (GL 40)	8.25 \pm 0.25 ^a	237.5 \pm 13.72	3.22 \pm 0.12 ^{ab}
		P= 0.23	

One way ANOVA with Duncan's post hoc test (SPSS).

The mean values with different superscripts differ significantly at $p < 0.05$

Table 2: The mean (\pm SE) haematological parameters (Hb, TEC, TLC, PCV and Platelet) of different groups comparison to Group I and II

GROUPS	Hb (g/dl)	Comparison with		TEC (10 ⁶ / μ l)	Comparison with	
		Group I	Group II		Group I	Group II
Group I (NC)	15.1 \pm 0.63 ^b	-	22.2	5.48 \pm 0.21 ^b	-	18.2
Group II (DC)	12.36 \pm 0.17 ^a	-18.1	-	4.57 \pm 12 ^a	-15.4	-
Group III (RC)	14.2 \pm 0.36 ^b	-6.0	14.9	5.22 \pm 23 ^b	-3.3	14.2
Group IV (GL 10)	14.48 \pm 0.24 ^b	-4.1	17.2	5.3 \pm 15 ^b	-1.9	16.0
Group V (GL 20)	14.08 \pm 0.33 ^b	-6.8	13.9	5.15 \pm 15 ^b	-4.4	12.9
Group VI (GL 40)	14.44 \pm 0.28 ^b	-4.4	16.8	5.4 \pm 0.09 ^b	-1.4	18.2

One way ANOVA with Duncan's post hoc test (SPSS).

The mean values with different superscripts differ significantly at $p < 0.05$

Table 2: (Cont...)

GROUPS	TLC (10 ³ / μ l)	Comparison to		PCV (%)	Comparison to		Platelet (10 ³ / μ l)	Comparison to	
		Group I	Group II		Group I	Group II		Group I	Group II
GROUP I (NC)	12.16 \pm 1.35 ^a	-	-24.8	45.3 \pm 1.88 ^b	-	22.2	431.1 \pm 22.94 ^b	-	42.1
GROUP II (DC)	16.17 \pm 0.2 ^b	33.0	-	37.08 \pm 0.52 ^a	-18.1	-	303.3 \pm 17.8 ^a	-29.6	-
GROUP III (RC)	12.84 \pm 2.02 ^a	5.7	-20.5	42.6 \pm 1.07 ^b	-6.0	14.9	410.01 \pm 21.31 ^b	-4.89	35.17
GROUP IV (GL 10)	13.01 \pm 1.13 ^{ab}	6.9	-19.6	43.44 \pm 0.72 ^b	-4.1	17.2	380.6 \pm 15.04 ^b	-11.71	25.48
GROUP V (GL 20)	12.72 \pm 0.29 ^a	4.6	-21.3	42.24 \pm 0.98 ^b	-6.8	13.9	390.8 \pm 35.63 ^b	-9.3	28.84
GROUP VI (GL 40)	12.38 \pm 0.35 ^a	1.8	-23.4	43.32 \pm 0.83 ^b	-4.4	16.8	401.2 \pm 49.23 ^b	-6.9	32.27

One way ANOVA with Duncan's post hoc test (SPSS).

The mean values with different superscripts differ significantly at $p < 0.05$

Table 3: The mean (\pm SE) biochemical parameters (AST, ALT, ALP, BUN, CRT, TP) in Comparison to Group I and II

GROUPS	AST(IU/L)	Comparison to		ALT (IU/L)	Comparison to		ALP(IU/L)	Comparison to	
		Group I	Group II		Group I	Group II		Group I	Group II
Group I (NC)	110.23 \pm 3.42 ^a	0.0	-41.7	73.1 \pm 3.025 ^a	0.0	-30.8	276.5 \pm 16.4 ^a	0.0	-44.9
Group II (DC)	189.77 \pm 10.42 ^c	71.6	0.0	105.00 \pm 3.31 ^b	44.5	0.0	501.83 \pm 88.07 ^b	81.5	0.0
Group III (RC)	153.9 \pm 8.23 ^b	39.6	-18.7	82.12 \pm 4.26 ^a	12.3	-22.2	385.16 \pm 36.22 ^{ab}	39.3	-23.2
Group IV (GL 10)	143.65 \pm 11.38 ^b	35.5	-21.0	84.2 \pm 4.34 ^a	14.6	-20.6	341.8 \pm 28.42 ^a	23.6	-31.9
Group V (GL 20)	146.4 \pm 11.40 ^b	30.3	-24.1	80.80 \pm 5.44 ^a	11.0	-23.2	331.93 \pm 17.97 ^a	20.0	-33.9
Group VI (GL 40)	147.402 \pm 6.69 ^b	32.8	-22.6	76.93 \pm 3.704 ^a	4.8	-27.5	332 \pm 27.37 ^a	20.1	-33.8

One way ANOVA with Duncan's post hoc test (SPSS).

The mean values with different superscripts differ significantly at $p < 0.05$

Table 3: (Cont...)

GROUPS	BUN (mg/dl)	Comparison to		CRT (mg/dl)	Comparison to		TP(g/dl)	Comparison to	
		Group I	Group II		Group I	Group II		Group I	Group II
Group I (NC)	10.97 \pm 0.81 ^a	-	-40.4	0.45 \pm 0.04 ^a	-	-47.1	5.58 \pm 0.09 ^a	-	29.5
Group II (DC)	18.42 \pm 1.83 ^b	67.9	-	0.85 \pm 0.05 ^b	88.9	-	4.31 \pm 0.39 ^c	-22.8	-
Group III (RC)	14.06 \pm 1.48 ^a	28.2	-23.7	0.48 \pm 0.04 ^a	6.7	-43.5	5.08 \pm 0.49 ^b	-9.0	17.9
Group IV (GL 10)	15.37 \pm 2.92 ^{ab}	16.5	-16.8	0.59 \pm 0.08 ^a	31.1	-30.5	4.9 \pm 0.08 ^b	-12.2	13.7
Group V (GL 20)	11.79 \pm 0.68 ^a	7.5	-36.0	0.55 \pm 0.04 ^a	22.2	-66.7	5.2 \pm 0.21 ^{ab}	-6.8	20.6
Group VI (GL 40)	12.44 \pm 2.01 ^a	13.4	-32.5	0.51 \pm 0.05 ^a	13.3	-40.	5.4 \pm 0.19 ^a	-3.2	25.3

One way ANOVA with Duncan's post hoc test (SPSS).

The mean values with different superscripts differ significantly at $p < 0.05$.

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