WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 12, 725-749. Research Article ISSN 2277– 7105

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF LILIUM CANDIDUM.L IN EXPERIMENTAL ANIMAL MODELS

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Article Received on
23 Sept. 2016,

Revised on 13 Oct. 2016,
Accepted on 03 Nov. 2016

DOI: 10.20959/wjpr201612-7420

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ABSTRACT

Lilium Candidum (Family liliaceae) commonly known as Madonna lilies is a vigorously growing in India. In the present study a pharmacognostics evaluation of the leaves was undertaken. In addition to the evaluation of physicochemical characteristics; preliminary Phytochemical parameters and pharmacological activities of aqueous extracts has been carried out. The aim of the present study was carried out with the objective of phytochemical screening and to evaluate the hepato protective activity of aqueous extract of L. candidum. Liver is the largest organ in the body which serves as a gland also. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. Any damage to the liver or impairment of its functions leads to injurious effects. Liver diseases (like jaundice) are the common ailments affecting mankind, though no remedy is available in allopathic at present. In the recent past years many medicinal plants are screened for their hepato protective activity and quite a few of them are already successful in entering the market, Hence the present study is planned to find out the hepato protective activity of Lilium Candidum using drug induced hepatotoxicity models like Paracetamol, CCL_4 and Acetaminophen induced methods. The rats were divided into five groups with six rats in each for three models. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals on the 3rd and 4th day. Group III and IV were treated with LCM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3rd and 4th day with hepatotoxic drugs was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day hepatotoxic

drugs was given 1h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of hepatotoxic drugs under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters. In hepatoprotective studies, the induced Diclofenac, CCL_4 and Acetaminophen toxicity elevated levels of serum marker enzymes ALT, AST, ALP and the level of BUN along with the decrease in total protein and albumin levels. It also increased the relative liver weight and decreased the level of liver total protein and GSH. The activity of catalase and GPx significantly decreased in diclofenac intoxicated animals. The pre-treatment of methanol extract of Lilium candidum at dose levels of 400 and 600 mg/kg had restored the ALT, AST, ALP and BUN levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg). The total protein, albumin, GSH levels and catalase, GPx activity increased significantly in the animals received pre-treatment of the LCM. The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $p < 0.01$ for ANOVA and $P < 0.05$ for t-test.

KEYWORDS: Lilium candidum, hepato protective activity, serum, total protein, albumin, Blood Urea Nitrogen, Alkaline Phosphate, Aspartate amino transferase, Alanine amino transferase.

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles.[1] Liver functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Additionally, it also handles the metabolism and excretion of drugs and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them.[2] Liver cells possess the antioxidant defence system consisting of antioxidants such as GSH, ascorbic acid and vitamin E and antioxidant enzymes such as SOD, catalase and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death.[3] The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic.[4] Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals and this is one of the major reasons why the liver is a target for toxic substances.[5] During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress[6] which leads to the hepatic damage.

SELECTION OF THE PLANT FOR PRESENT STUDY

When selecting a plant for pharmacological activities, four basics methods are usually followed:

a) Random choice of plant species

b) Choice based on ethnomedical use

c) Follow up of existing literature on the use of the species

d) Chemotaxonomic approaches

Comparison of the four methods showed that the choice based on folklore has given about 25% more positive leads than other methods. Based on the second and third approach, selection of the plant has been made in the present work. In light of the above context, *Lilium candidum*(LC) flowers were selected for the study.

Lilium candidum L. is also called "white Madonna lily", is well known in folk medicine for the treatment of burns, ulcers, inflammations and for healing wounds. *Lilium candidum L.* extract contains various biologically active compounds. As the antimutagenic activity of natural compounds often correlates with antioxidant effects and contents of phytochemical substances from the flavonoids group, our hypothesis is that the LC extract, which is rich in flavonoids and with pronounced antioxidant activity, could possess bioprotective potential.

Considering the above, the objectives set forth are:

- Review of Literature for;

Reported anti-inflammatory activity of some medicinal plants

Reported hepatoprotective activity of some medicinal plants

- Physicochemical study of *Lilium candidum* flowers
- Hepatoprotective study of *Lilium candidum* flowers
- Toxicity study of *Lilium candidum* flowers.

The aim of the study to evaluate hepatoprotective *Lilium Candidum* flowers extract.

1. To prepare methanolic extract of *Lilium Candidum*.
2. To perform the phytochemical screening of LCM.
3. To determine the hepatoprotective activity in different models:
   i. Carbon Tetrachloride induced liver damage in rats.
   ii. Diclofenac induced liver damage in rats.
   iii. Acetaminophen induced liver damage in rats.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The fresh flowers of *Lilium candidum* were collected from the local market of Hyderabad.

PREPARATION OF THE EXTRACT

The flowers were washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was first defatted with petroleum ether and then extracted with methanol by using Soxhlet apparatus (Lin et al., 1999). The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The percentage yield of methanol extract was 36%. The methanol extract of *Lilium candidum* (LCM) was used for the entire study.

PHYTOCHEMICAL ANALYSIS

Preliminary chemical tests were carried out for methanolic extract to identify different phyto-constituents.

Alkaloids

The crude powder and methanol extract of *Lilium candidum* flowers was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity but no flocculation was observed and a (+++) score was recorded if heavy precipitate or flocculation was observed.

3.2. Flavonoids

Shinoda test

The presence of flavonoids was estimated by Shinoda test. The crude powder and methanol extract of *Lilium candidum* flowers were treated with few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within few minutes indicated the presence of flavonoids.

Alkaline reagent test

The crude powder and methanol extract of *Lilium candidum* flowers was treated with few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

Cardiac glycosides

Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and methanol extract of *Lilium candidum* flowers was treated with 1 ml mixture of 5% $FeCl_3$ and glacial acetic acid (1:99 v/v). To this solution, few drops of concentrated H_2SO_4 were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides.

Phlobatannins

The crude powder and methanol extract of Wood *Lilium candidum* fordia fruticosa flowers was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatannins.

Saponins

The presence of saponins was determined by Frothing test. The crude powder and methanol extract of *Lilium candidum* flowers was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5 cm indicated the presence of saponins.

Steroids

Liebermann-Burchard reaction was performed for the presence of steroids. A chloroformic solution of the crude powder and methanol extract of *Lilium candidum* flowers was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

Tannins

The crude powder and methanol extract of *Lilium candidum* flowers was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue color indicated the presence of tannins.

Triterpenes

Chloroform extract of the crude powder and methanol extract of *Lilium candidum* flowers was treated with concentrated sulphuric acid (H_2SO_4). Appearance of reddish brown ring indicated the presence of triterpenes.

ANIMALS

Wistar albino rats of both sexes (180-220 g) were used for the study. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were kept at the animal house of Department of Pharmacology. The animals were facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Baroda. Amruth Brand rat & mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA recognized local ethical committee.

SELECTION OF THE DOSES FOR ANIMAL STUDY

The dose considered for the experiment on rats was obtained from conversion of human dose of *Lilium candidum* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence the calculated dose for the rats (considering human dose 5 g/kg) is 450 mg/kg. Thus, hepatoprotective activity was done at two different doses 400 and 600 mg/kg body weight. Acute toxicity was done at three different doses 450, 1800, and 3600 mg/kg body weight.

HEPATOPROTECTIVE STUDIES

Diclofenac induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received diclofenac (50 mg/kg i.p.) on the 3rd and 4th day.

Group III (LCM-400) and IV (LCM-600) were treated with LCM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3rd and 4th day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4th day diclofenac (50 mg/kg i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of diclofenac under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.

Carbon tetrachloride induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) On the 3rd and 4th day. Group III (LCM-400) and IV (LCM-600) were treated with LCM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 3rd and 4th day CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 3rd and 4^{th} day CCl_4 (2 ml/kg, 1:1 in olive oil, i.p.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the last injection of CCl_4 under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters.

Acetaminophen induced hepatotoxicity

The rats were divided into five groups with six rats in each. Group I (Control) served as normal and received the vehicle alone (Sterile distilled water, 10 ml/kg, p.o.) for 5 days. Group II (Toxin control) animals received acetaminophen (3 g/kg, p.o.) on the 4th day. Group III (LCM-400) and IV (LCM-600) were treated with LCM at a dose level of 400 mg/kg and 600 mg/kg body weight p.o. per day respectively for 5 days and on the 4th day acetaminophen (3 g/kg, p.o.) was given 1 h after the treatment of the extract. Group V (Standard) was treated with standard drug silymarin (100 mg/kg p.o.) for 5 days and on the 4th day acetaminophen (3 g/kg, p.o.) was given 1 h after the treatment of the drug. The animals were sacrificed 48 h after the dose of acetaminophen under mild ether anesthesia. The blood was collected and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to estimate various biochemical parameters. In above three hepatoprotective models, various in vivo antioxidant parameters were estimated from liver.

Preparation of liver homogenate

The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the liver was homogenized in chilled Tris-HCl buffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5,000 rpm for 10 min, supernatant was collected and used for analysis.

Biochemical analysis from serum

The absorbance of all the biochemical parameters was measured in a UV–VIS Spectrophotometer - 1601.

Estimation of total protein content

The serum total protein was estimated by modified Biuret method using the total protein test kit.

3.0 ml of Reagent I was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at 37ºC for 5 minutes. The absorbance was read at 578 nm.

Estimation of albumin content

3.0 ml of albumin reagent (Reagent I) was added to all the test tubes. Thereafter, 0.03 ml serum was added for the test and 0.03 ml Reagent II was added for the standard, while in blank 0.03 ml of purified water was added. They were then mixed well and incubated at room temperature for 1 min. The absorbance was read at 630 nm.

Estimation of blood urea nitrogen (BUN) content

The serum blood urea nitrogen was estimated by Enzymatic Urease (Berthelot) method (Fawcett and Scott, 1960) using Urea Berthelot test kit.

1.5 ml Solution I was added to clean test tubes. 0.01 ml serum was added for the test and 0.01 ml Reagent III was added for the standard. It was then mixed well and incubated at 37ºC for 3 min; then 1.5 ml of Solution II was added. It was then mixed well and incubated at 37ºC for 5 min. The absorbance was read at 578 nm against reagent blank.

Estimation of alkaline phosphatase (ALP) activity

All the test tubes were marked properly as blank (B), standard (S), control (C) and test (T). 0.5 ml of working buffered substrate was added in clean tubes. 1.5 ml of purified water was added in all the tubes. They were mixed well and incubated at 37°C for 3 min. 0.05 ml of serum was added in test (T), 0.05 ml of reagent III (Phenol standard) was added in standard (S) and 0.05 ml of purified water was added in blank (B) tubes. All the tubes were mixed well and incubated at 37°C for 15 min. 1 ml of reagent II was added in all the tubes. 0.05 ml of serum was added in control (C). All the tubes were mixed well and absorbance was read at 510 nm. Serum alkaline phosphatase activity is expressed as KA units.

Estimation of aspartate aminotransferase (AST) activity

The serum aspartate aminotransferase was estimated by the method of Reitman and Frankel (1957) using AST test kit.

0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 60 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505nm.

Estimation of alanine aminotransferase (ALT) activity

The serum alanine aminotransferase was estimated by the method of Reitman and Frankel (1957) using ALT test kit (Span Diagnostics Ltd.).

Reagents

Reagent I: Buffered alanine - α-KG substrate, pH 7.4

Reagent II: DNPH (2,4- Dinitrophenyl hydrazine) colour reagent

Reagent III: Sodium hydroxide, 4 N

Reagent IV: Working Pyruvate Standard, 2 mM

Solution I: Dilute 1 ml of Reagent III up to 10 ml with purified water.

Procedure: 0.25 ml of Reagent I was added in clean test tubes and incubated at 37ºC for 5 minutes. 0.05 ml of serum was added in the test, 0.05 ml Reagent IV was added in the standard and 0.05 ml distilled water was added in the blank. They were mixed well and incubated at 37ºC for 30 minutes. Thereafter, 0.25 ml of Reagent II was added to all the tubes, mixed well and allowed to stand at room temperature for 20 min. Then 2.5 ml of Solution I was added to all the tubes, mixed well and allowed it to stand at room temperature for 10 min. The absorbance of blank, standard and test were read at 505 nm.

Acute Toxicity Study

Acute oral toxicity (Ryu et al., 2004), study was performed as per OECD-404 guidelines (1987). 10 rats/group (5 males and 5 females) were used for the study. Group 1 was control group and other three groups were that of WFM at different doses (450, 1800 and 3600 mg/kg body weight). Single dose of the extract was administrated orally to each animal. Signs of toxicity, body weight, feed and water consumption of each animal was observed every day for 14 days.

Statistical Analysis

The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Student's t-test. Values are considered statistically significant at $p < 0.01$ for ANOVA and $P < 0.05$ for t-test.

RESULTS AND DISCUSSION

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The results of qualitative phytochemical analysis of the crude powder and the methanol extract of *Lilium candidum* flowers is shown in Table ---.

Table ---: Preliminary qualitative phytochemical analysis of *Lilium candidum* flowers

Phytochemical	Test	Methanolic extract
Alkaloids	Dragendorff 's test	+
	Mayers test	+
	Wagners test	+
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Cardiac glycosides	Keller-kilianni test	-
Phlobotannins	HCl test	+
Saponins	Frothing test	+
Steroids	Libbermann-Burchard test	-
Tannins	$FeCl_3$ test	+
Triterpenes	H_2SO_4 test	+

(-): absent, (+): present.

In methanol extract maximum amount of tannins, alkaloids, Flavonoids, phlobotannins, saponins and triterpenes were present. Cardiac glycosides and steroids were absent.

HEPATOPROTECTIVE STUDIES

Diclofenac induced hepatotoxicity

The results of serum biochemical parameters in pre-treatment of LC with respect to induction of hepatotoxicity using diclofenac are shown in Figure 2. The level of total protein and albumin depleted in the group treated with diclofenac (toxin control) and were significantly decreased ($P < 0.001$) when compared with the normal control group. The BUN and ALP levels increased significantly ($P < 0.01$, $P < 0.001$ respectively) in the group treated with diclofenac. The administration of diclofenac markedly increased serum AST and ALT levels which were significant as compared to normal control group ($P < 0.05$, $P < 0.01$ respectively).

The groups that received the pre-treatment of LCM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters. The extract at dose levels of 400 and 600 mg/kg exhibited significant increases ($P < 0.05$) in the serum total protein level as compared to toxin control group. The albumin level in lower as well as in higher dose group increased significantly ($P < 0.01$, $P < 0.001$ respectively) as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin. The BUN level decreased in both the dose groups significantly ($P < 0.05$) as compared to toxin control group. The ALP level also significantly decreased in LCM-400 ($P < 0.05$) as well as in LCM- 600 group ($P < 0.001$). In LCM-600 group, the level of ALT and AST significantly decreased ($P < 0.05$), the result was comparable to that of standard group.

The results of relative liver weight, liver total protein, GSH and antioxidant enzymes in diclofenac induced hepatotoxicity are given in Figure 3. The relative liver weight in toxin control group increased significantly ($P < 0.001$) as compared to normal control group. The total protein and GSH levels from the liver homogenate decreased significantly ($P < 0.001$, $P < 0.01$ respectively) in toxin control group. The catalase (CAT) and GPx activity in the toxin control group was also significantly ($P < 0.001$, $P < 0.05$ respectively) depleted as compared to the normal control group. The mean relative liver weight decreased significantly in LCM-400 ($P < 0.001$) and LCM-600.

Figure 1

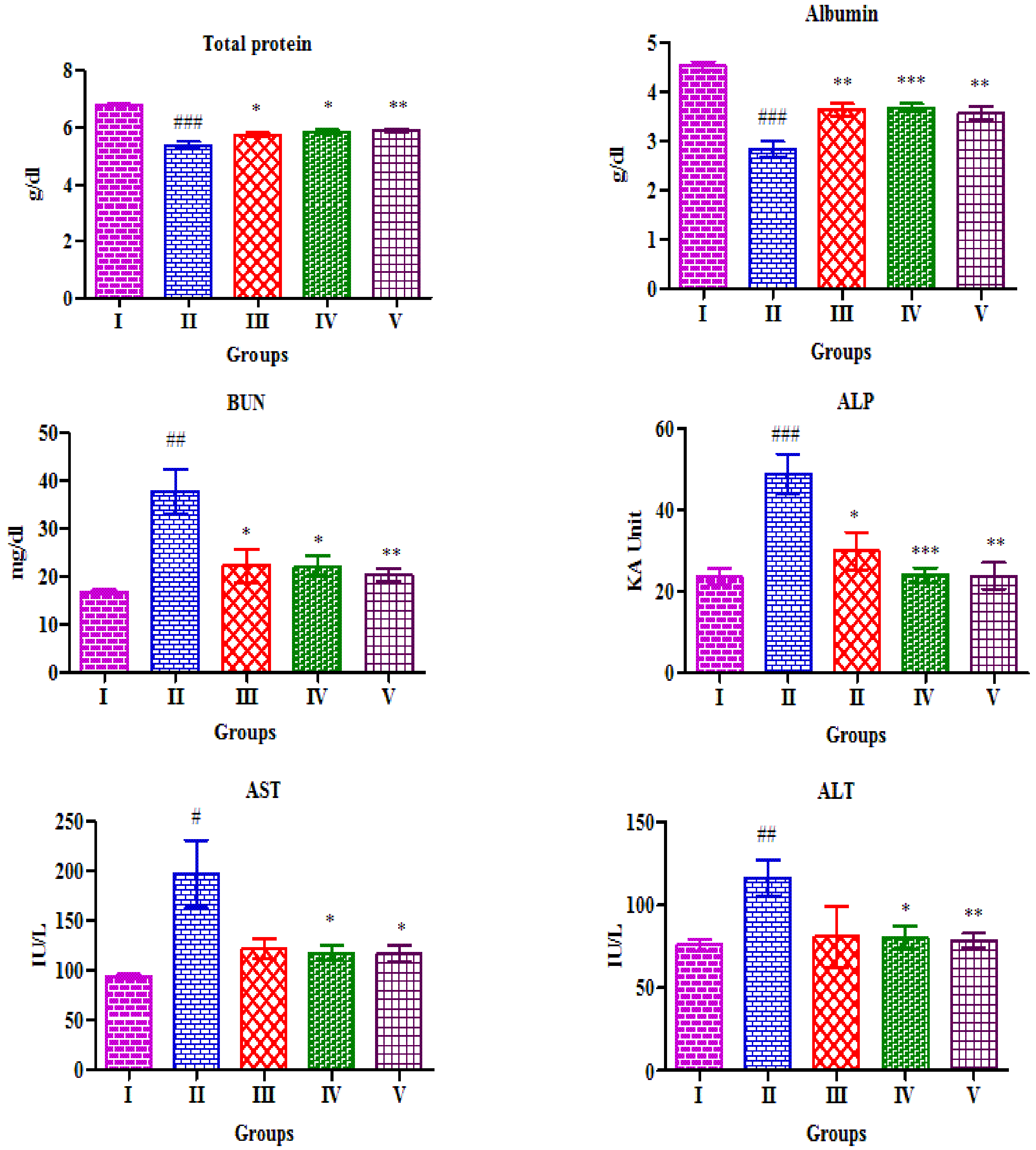


Figure 1: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: LCM-400 mg/kg + diclofenac, Group IV: LCM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM, (n = 6). #P < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with toxin control group.

Figure 2

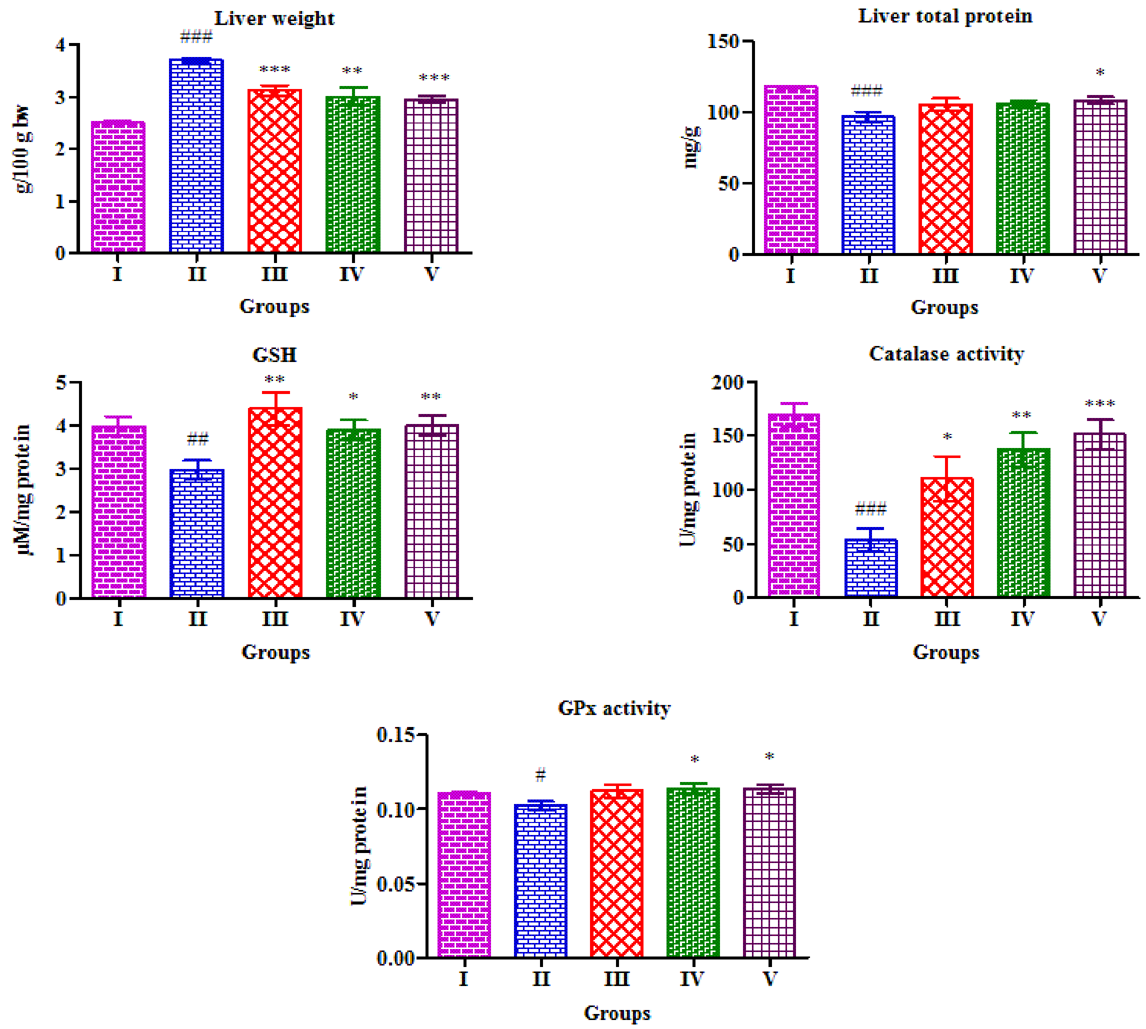


Figure 2: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: LCM-400 mg/kg + diclofenac, Group IV: LCM-600 mg/kg + diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p <0.01as compared with toxin control group. (P < 0.01) treated group as compared to the toxin control group. The result of the higher dose group was comparable to the standard drug treated group (P < 0.001). The total protein and GSH levels from liver homogenate in LCM treated groups elevated, but total protein level was not significant. However, pretreatment with LCM significantly recovered the diclofenac induced GSH depletion in lower and higher dose group (P < 0.01, P < 0.05 respectively). The catalase and GPx activity increased at both the dose levels; at higher dose LCM exhibited good activity (P < 0.01, P < 0.05 respectively). GPx activity of LCM-600 group was similar to that of standard drug treated group.

Carbon tetrachloride induced hepatotoxicity

The results observed from serum biochemical parameters in pre-treatment of LCM with respect to induction of hepatotoxicity using CCl_4 are given in Figure 4. A marked reduction in total protein and albumin levels was observed in the group treated with CCl_4 and they were significantly decreased ($P < 0.05$) when compared with the normal control group. The BUN and ALP levels increased in the group treated with CCl4 but not to a significant level. Rats treated with CCl_4 (toxin control) developed significant liver damage and it was well indicated by elevated levels of hepato specific enzymes like AST ($P < 0.01$) and ALT ($P < 0.001$) in serum. The groups received the pre-treatment of LCM at dose levels of 400 and 600 mg/kg body weight significantly controlled the change in the biochemical parameters. The extract at dose levels of 400 and 600 mg/kg exhibited significant increase ($P < 0.01$, $p < 0.05$ respectively) in the serum total protein level as compared to toxin control group and the effect was comparable with the standard group ($P < 0.01$) treated with silymarin (Sily-100). The albumin level also increased in drug treated groups but not to a significant level. The level of BUN was reduced in both the dose of LCM and standard drug treated groups, but it was not significant. The ALP ($P < 0.05$), AST ($P < 0.01$) and ALT ($P < 0.01$) levels significantly decreased in LCM-400 group as compared to toxin control group. LCM-600 group also showed significant decreased ($P < 0.05$) AST and ALP levels.

The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in CCl_4 induced hepatotoxicity are given in Figure 5. The relative liver weight in toxin control group increased significantly ($P < 0.001$) as compared to normal control group. The total protein level in liver decreased significantly ($P < 0.001$) in toxin control group. The level of GSH in toxin control group decreased, but it was non significant. The catalase (CAT) and GPx activities in the toxin control group depleted significantly ($P < 0.05$, $P < 0.001$ respectively) as compared to the normal control group. The mean relative liver weight in LCM at both the doses was slightly elevated as compared to the toxin control group. The total protein level in liver, in LCM treated as well as in the standard drug treated group, increased significantly ($P < 0.001$) as compared to toxin control group. GSH level increased significantly ($P < 0.01$) at higher dose as compared to toxin control group. Catalase activity increased at both the dose levels though not significantly, while in silymarin group, catalase activity decreased. Administration of LCM did not display effect of increase in the GPx activity.

Figure 3

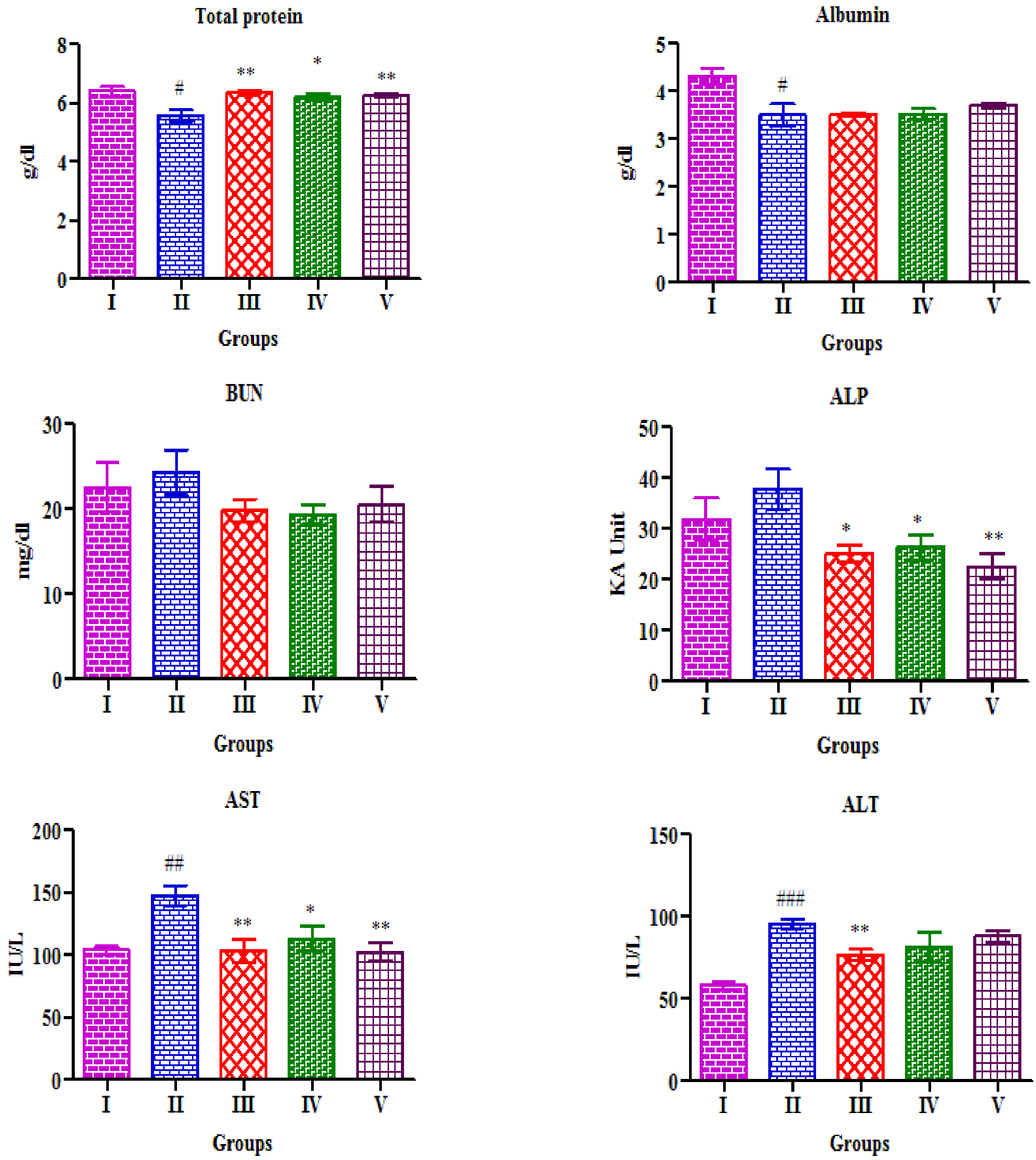


Figure 3: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: LCM-400 mg/kg + CCl_4, Group IV: LCM- mg/kg + CCl_4, Group V: Silymarin-100 mg/kg + CCl_4. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with toxin control group.

Figure 4

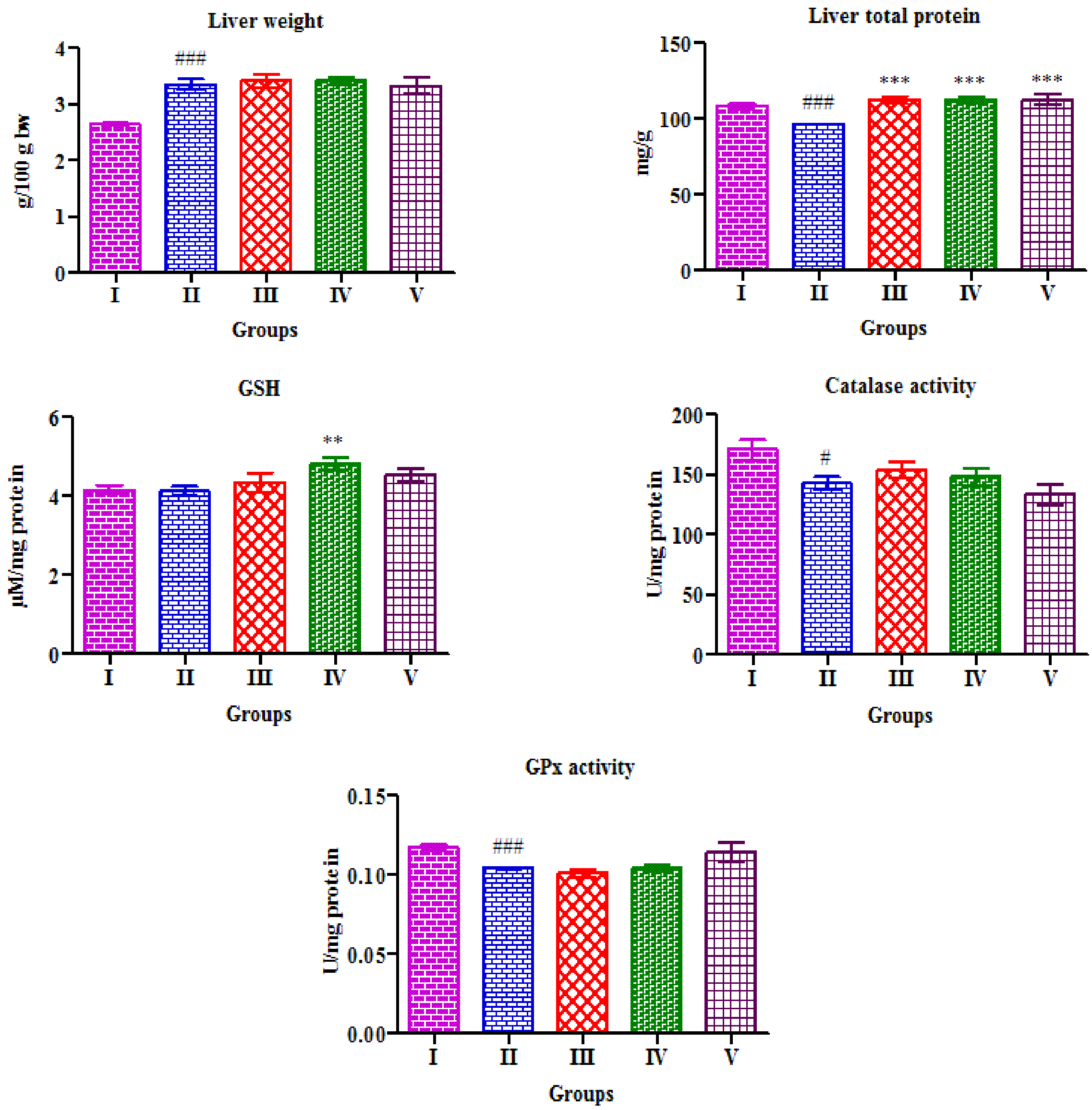


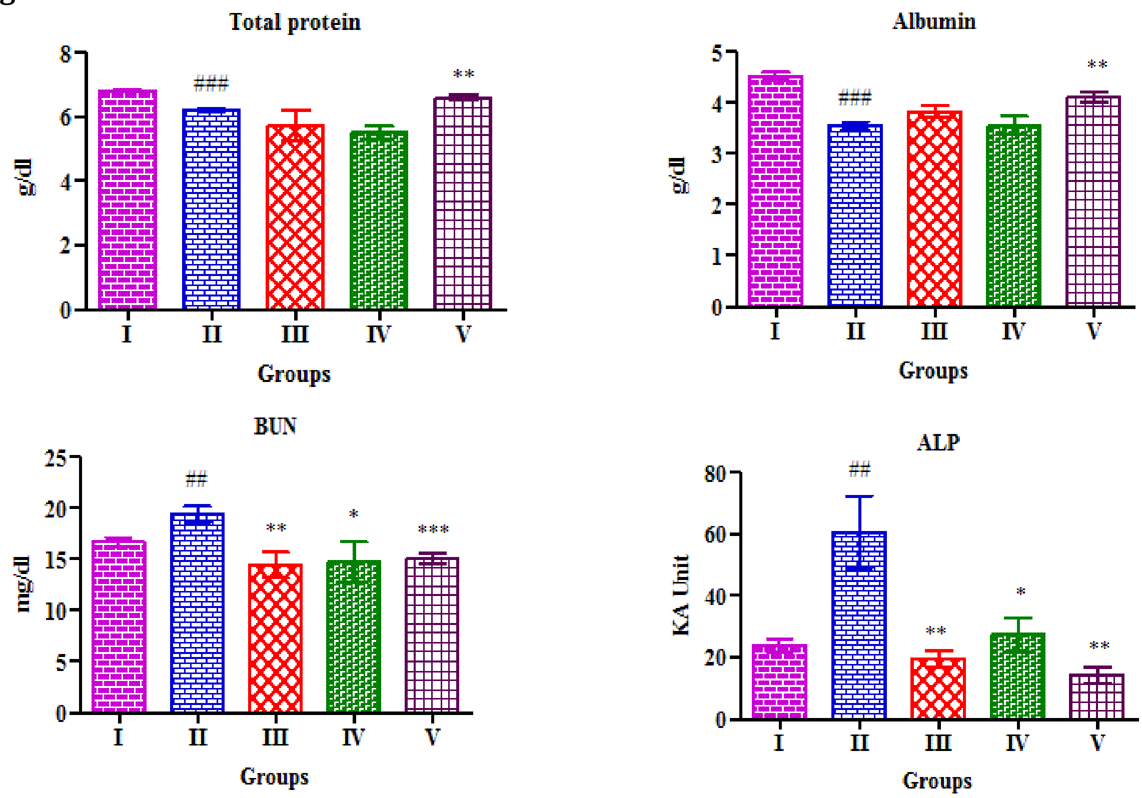
Figure 4: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: LCM-400 mg/kg + CCl_4, Group IV: LCM-600 mg/kg + CCl_4, Group V: Silymarin-100 mg/kg + CCl_4. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with toxin control group.

Acetaminophen induced hepatotoxicity

Oral administration of Acetaminophen (APAP) caused significant liver damage as evidenced by altered biochemical parameters (Figure 6). APAP significantly (P < 0.001) decreased serum levels of total protein and albumin as compared to normal control group. APAP significantly (P < 0.01) enhanced BUN, ALP, AST and ALT levels in the blood circulation; about 3-fold increase was observed in AST and ALT levels in serum.

Treatment with LCM did not exhibit potential effect on recovery of total protein and albumin levels; while in standard drug treated group, the level of total protein and albumin levels increased significantly (P < 0.01). The BUN and ALP levels also decreased significantly in lower as well as higher dose of LCM (P < 0.01, P < 0.05 respectively) as compared to toxin control group. 400 and 600 mg/kg of LCM treated group showed significant (P < 0.001, P < 0.01 respectively) decrease in AST level as compared to toxin control group. The result of AST was similar to that of the standard drug treated group (P < 0.001). ALT level decreased in LCM treated groups towards normalization though not significantly. The result of relative liver weight, liver total protein, GSH and antioxidant enzymes in APAP induced hepatotoxicity are given in Figure 7. The administration of APAP significantly increased the liver weight (P < 0.001) as compared to normal control group. Significant decreased level was observed in hepatic total protein (P < 0.001). The administration of APAP significantly decreased the hepatic non-enzymatic antioxidant GSH contents (P < 0.05). The treatment of LCM decreased liver weight significantly (P < 0.05) at both the dose levels as compared to toxin control group.

Figure 5



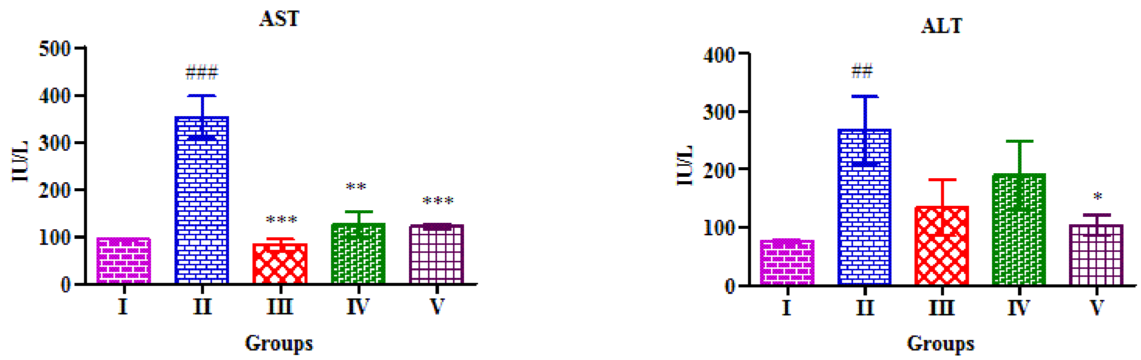
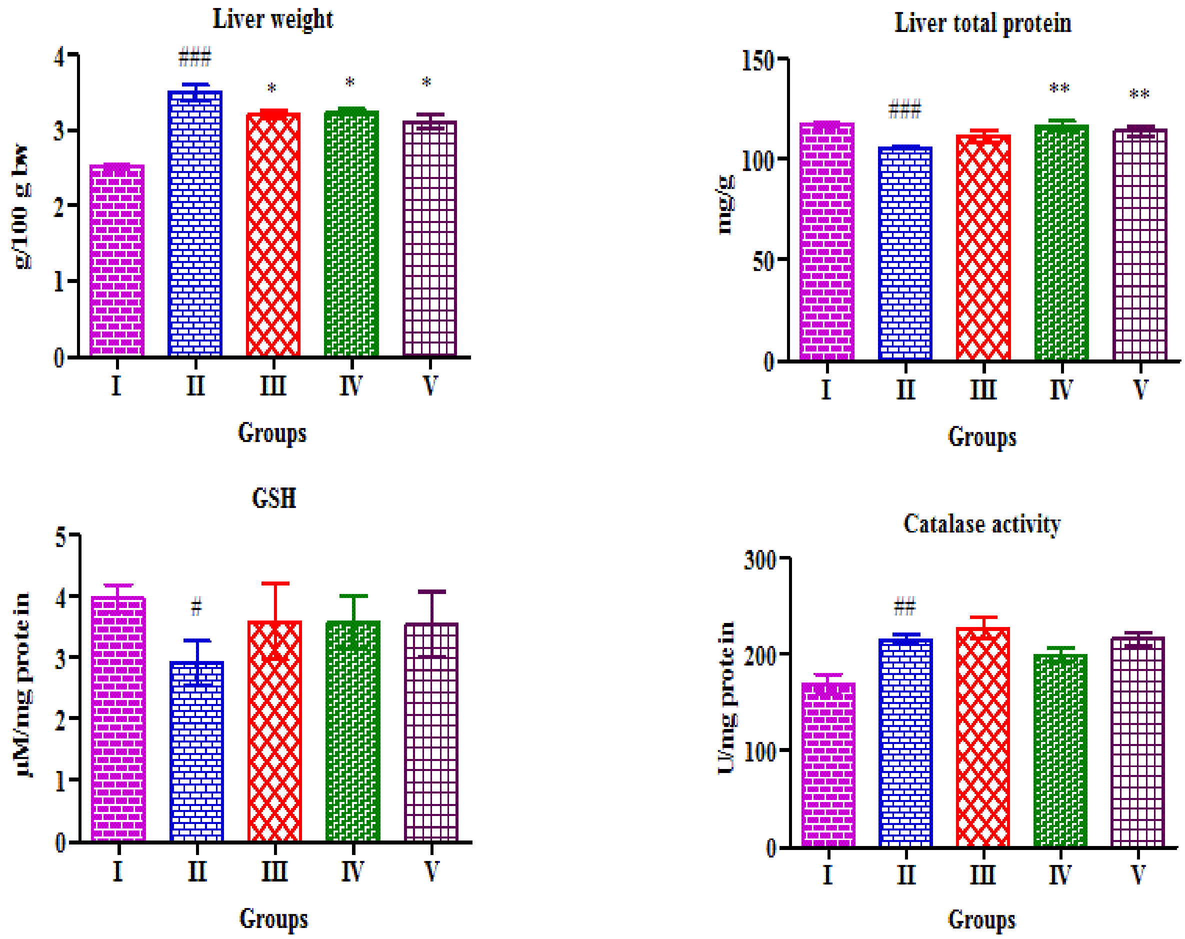


Figure 5: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: LCM-400 mg/kg + APAP, Group IV: LCM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with toxin control group.

Figure 6



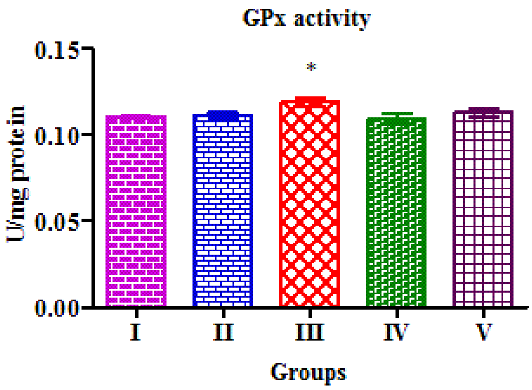


Figure 6: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: LCM- 400 mg/kg + APAP, Group IV: LCM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + APAP. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p < 0.01 as compared with control group.

In higher dose, the level of hepatic total protein increased significantly (P < 0.01). The hepatoprotective efficacy of the LCM-600 was comparable with that of standard drug silymarin. LCM treatment enhanced the production of GSH towards normal control, but not to a significant level. Administration of APAP did not diminish the antioxidative status of hepatic catalase and GPx activity.

ACUTE TOXICITY STUDY

In acute toxicity study, no adverse reactions or mortality were observed after administration of LCM (450, 1800 and 3600 mg/kg bw) and no behavioral changes were observed during the entire period of experimentation. Some alteration was noticed in daily feed and water intake in both male and female rats treated with single dose of extract as well as in control animals. As compared to the control group, drug treated groups had several consecutive days of reduced/increased feed and water consumption at different times in the study. These periods of reduced/increased feed and water intake were not significant to the overall feed and water consumption rates. Individual body weights were recorded daily during the experimental period. Mean body weight gains were calculated for each group. In control and LCM treated groups, body weight of animals slightly increased during experimental period, but the increase was not significant.

CONCLUSION

In physicochemical analysis, crude powder and methanol extract of *Lilium candidum* flowers were free from heavy metals. In qualitative phytochemical analysis tannins and alkaloids were present in higher amount, while cardiac glycosides and steroids were totally absent. In quantitative analysis of phytoconstituents, total phenol content was higher than flavonoid content. Hence, the determination of pharmacognostical and phyto-physicochemical profile of *Liliumcandidum*. L flowers may be useful to supplement information in respect to its identification, authentication and standardization of herbal drugs. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at herbal industrial level in the coming days. In hepatoprotective studies, the induced diclofenac toxicity elevated levels of serum marker enzymes ALT, AST, ALP and the level of BUN along with the decrease in total protein and albumin levels. It also increased the relative liver weight and decreased the level of liver total protein and GSH. The activity of catalase and GPx significantly decreased in diclofenac intoxicated animals. The pre-treatment of methanol extract of *Lilium candidum* at dose levels of 400 and 600 mg/kg had restored the ALT, AST, ALP and BUN levels towards normalization and the effects were comparable with standard drug (Silymarin 100 mg/kg).

The total protein, albumin, GSH levels and catalase, GPx activity increased significantly in the animals received pre-treatment of the LCM. In CCl_4 and acetaminophen induced hepatotoxicity models, the serum biochemical parameters and liver antioxidants were altered when animals were intoxicated with CCl_4 and acetaminophen. The treatment with LCM restored the level of serum biochemical parameters as well as liver antioxidants in both the animal models. The administration of acetaminophen and LCM did not have any effect in serum total protein level, catalase and GPx activity. In acute toxicity study, the methanol extract of *Lilium candidum* flowers had no mortality and observable acute toxic effect on the experimental animals and therefore can be considered as non-toxic. However, acute toxicity data sometimes is of limited clinical application since accumulative toxic effect may not be seen in short period with a single dose application. Hence, sub acute and chronic evaluation of the extract should be carried out in evaluating the safety profile of *Lilium candidum*. These studies have shown that the methanol extract of flowers of *Lilium candidum* contain some active ingredients with the potential of being good hepatoprotective agents. For that, further study for detailed investigation of the mechanism of action of LCM is needed.

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