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Research

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *LILIUM CANDIUM.L* IN ANIMAL MODELS

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Abstract

Lilium Candium (Family liliaceae) commonly known as Madonna lilies is a vigorously growing in India. In the present study a pharmacognostic 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 hepatoprotective activity of aqueous extract of *L.candium*.

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 hepatoprotective activity and quite a few of them are already successful in entering the market, Hence the present study is planned to find out the hepatoprotective activity of *Lilium Candium* using drug induced hepatotoxicity models like Paracetamol, CCL_4 and Acetamenophenon 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 Acetamenophenon 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 candium,* hepatoprotective activity, serum, total protein, albumin, Blood Urea Nitrogen, Alkaline Phosphate, Aspartate amino trasferase, Alanine amino transferase.

INTRODUCTION

1.1 INTRODUCTION TO LIVER

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.

1.1.1. Liver diseases

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Some of the commonly known disorders are viral hepatitis, alcohol liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver disease; drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year[7].

According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3–4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually[8]. Depending on the duration of the disease the liver diseases are classified as acute or chronic. If the disease does not exceed six months it is considered as acute liver disorder while diseases of longer duration are classified as chronic. Acute viral hepatitis and drug reactions account for the majority of cases of acute liver disease. Hepatitis A and B are the commonest causes of viral hepatitis in Europe and hepatitis E is common in India. Hepatitis C is not usually recognised as an acute infection because it rarely causes jaundice at this stage.

Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer[9]. Chronic hepatitis or long term intoxification can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis and necrosis, and eventually may progress to hepatoma.

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, schistosomiasis, etc; or chemical factors such as CCl_4 administration, alcohol intake, etc. were known to contribute to liver fibrosis. Hepatic fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholestatic and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes and causes apoptosis and/or necrosis [10].

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, nonalcoholic fatty liver disease, and chronic hepatitis C [11&12]. In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality[13].

Drug/chemical-mediated hepatic injury is the common sign of drug toxicity[14] and accounts for greater than 50% of acute liver failure cases. Hepatic damage is the largest obstacle to the development of drugs and is the major reason for withdrawal of drugs from the market [15]. Drug-induced liver disease can be predictable (high incidence and dose-related) or unpredictable (low incidence and may or may not be dose-related). Unpredictable reactions, also referred to as idiosyncratic, can be viewed as either immune-mediated hypersensitivity or nonimmune reactions. Most potent predictable hepatotoxins are recognized in the animal testing or clinical phase of drug development.

MATERIALS AND METHODS

1. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

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

2. 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.

RESULTS

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	Dragandroffs 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.

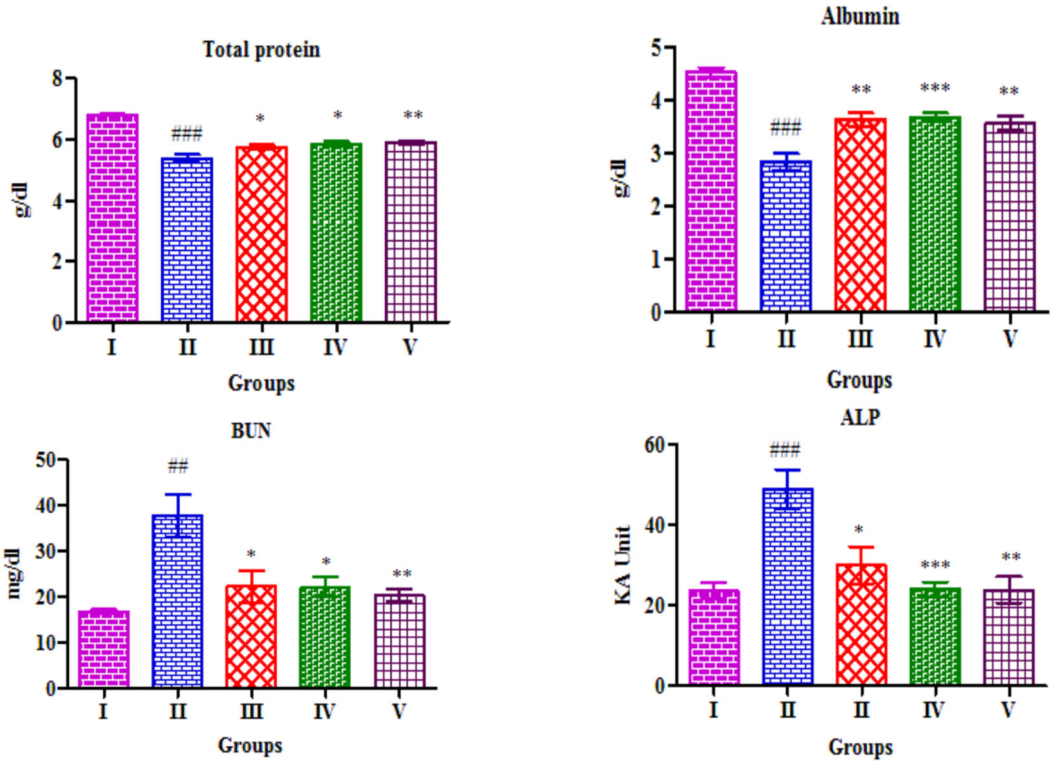
HEPATOPROTECTIVE STUDIES

4.6.1. 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.



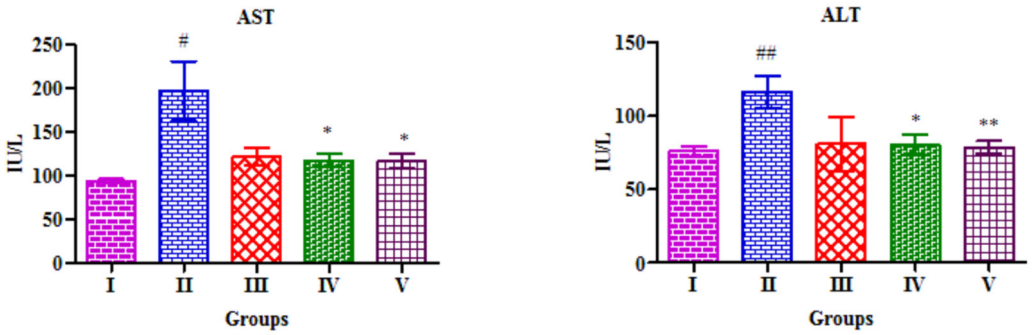


Figure 2: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: LCM-400 mg/kg + diclofenac, Group IV: LCM-600 mg/kg + diclofenac, 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.

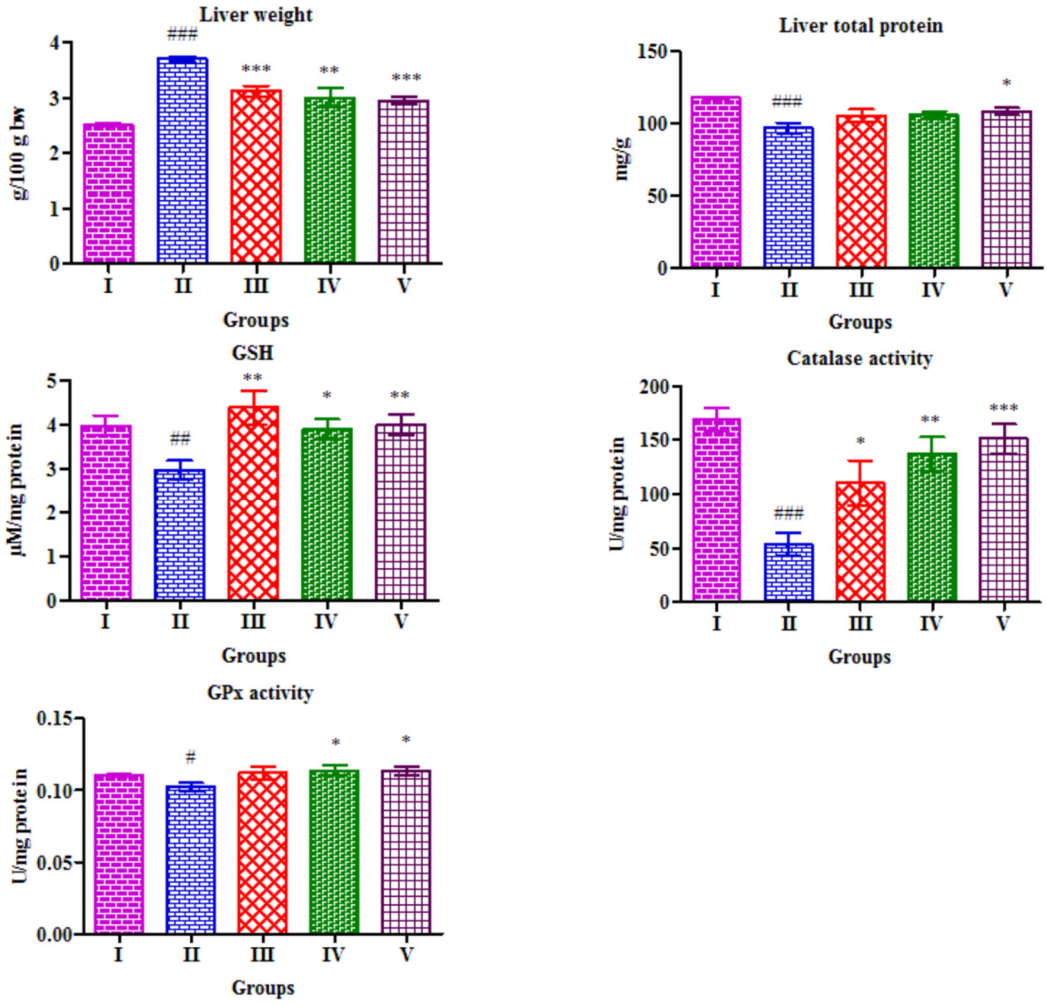


Figure 3: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in diclofenac (50 mg/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control diclofenac, Group III: LCM-400 mg/kg + diclofenac, Group IV: LCM-600 mg/kg +

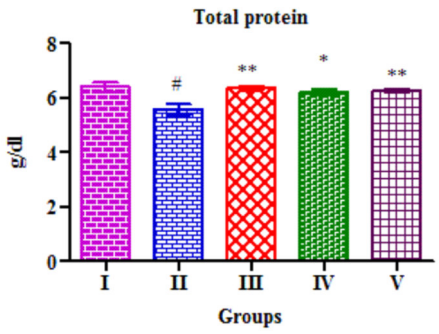
diclofenac, Group V: Silymarin-100 mg/kg + diclofenac. Results are expressed as mean ± SEM, (n = 6). #p < 0.05, ##p < 0.01, ###p < 0.001 as compared with normal control group; *p < 0.05, **p < 0.01, ***p <0.01as compared with toxin control group. (P < 0.01) treated group as compared to the toxin control group. The result of the higher dose group was comparable to the standard drug treated group (P < 0.001).

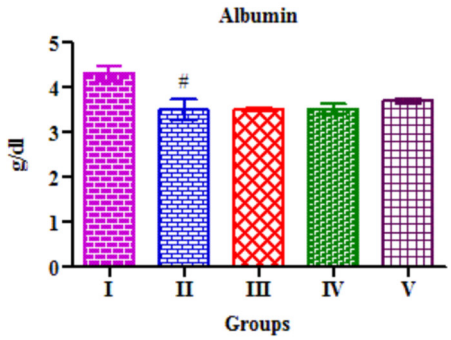
The total protein and GSH levels from liver homogenate in LCM treated groups elevated, but total protein level was not significant. However, pretreatment with LCM significantly recovered the diclofenac induced GSH depletion in lower and higher dose group (P < 0.01, P < 0.05 respectively). The catalase 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.

4.6.2. 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.





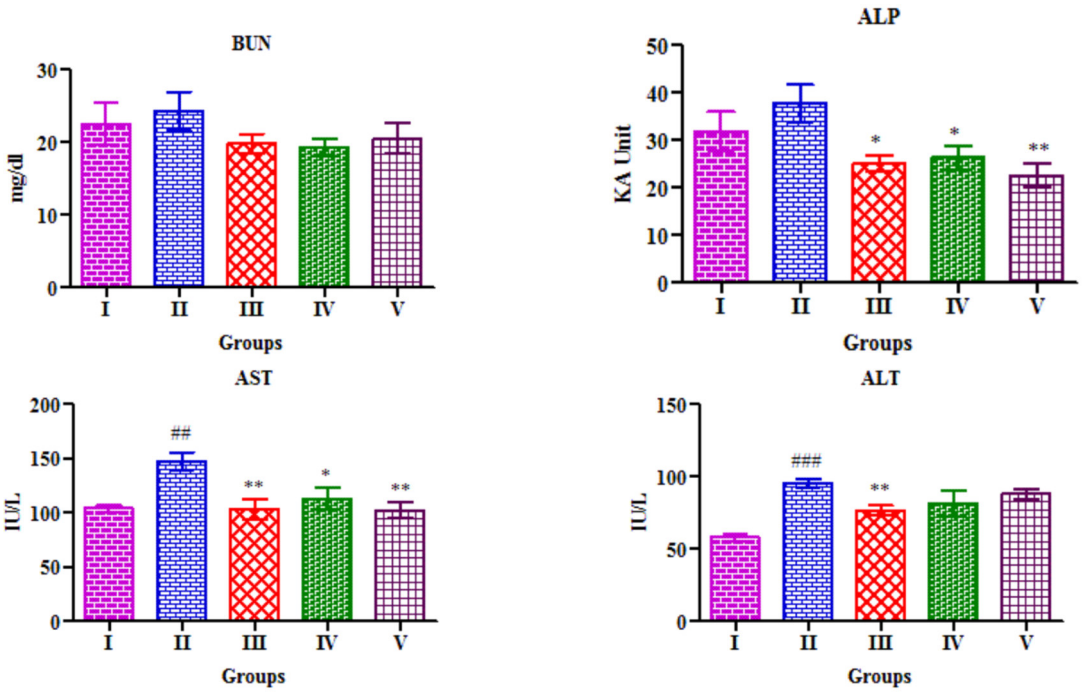
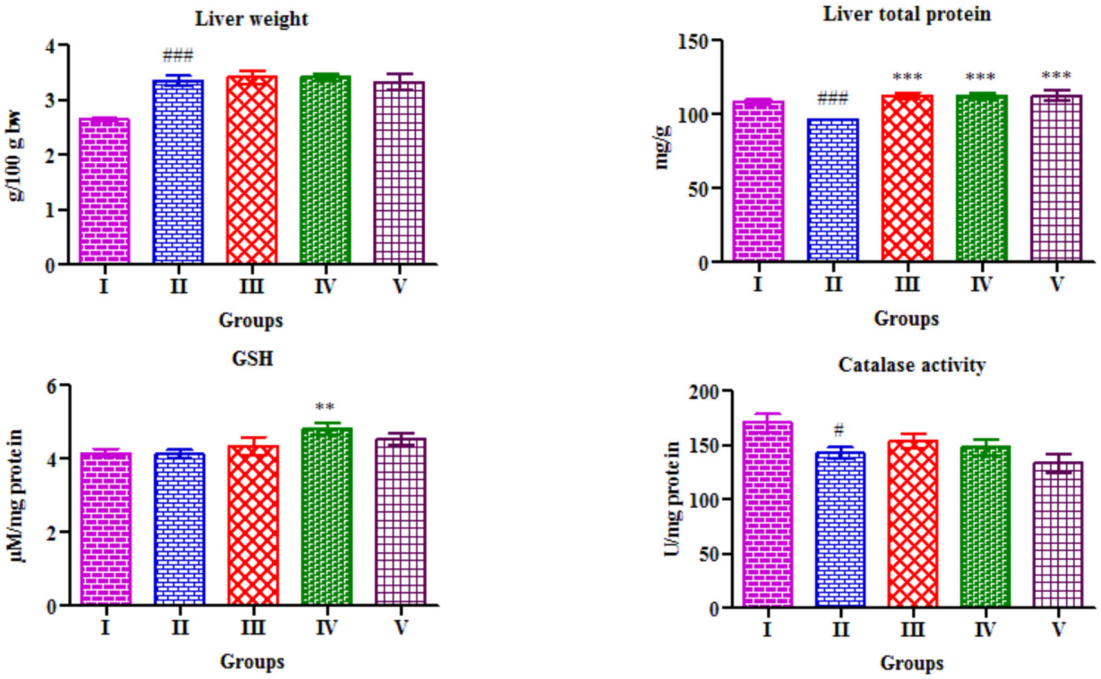


Figure 4: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: LCM-400 mg/kg + CCl_4, Group IV: LCM- mg/kg + CCl_4, Group V: Silymarin-100 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.



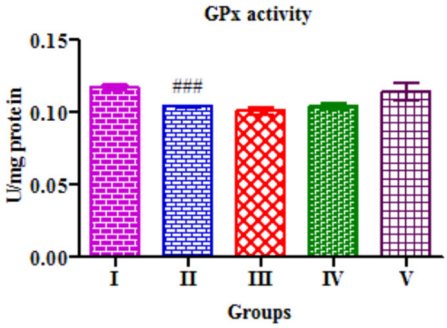


Figure 5: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in CCl_4 (2 ml/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control CCl_4, Group III: LCM-400 mg/kg + CCl_4, Group IV: LCM-600 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.

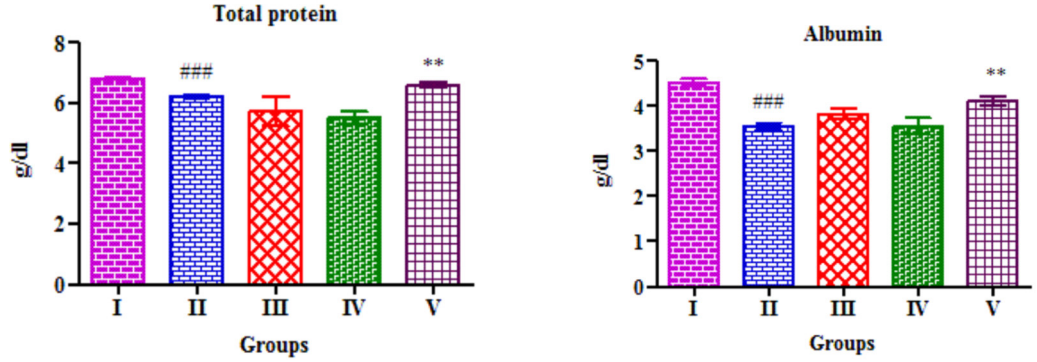
4.6.3. Acetaminophen induced hepatotoxicity

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.



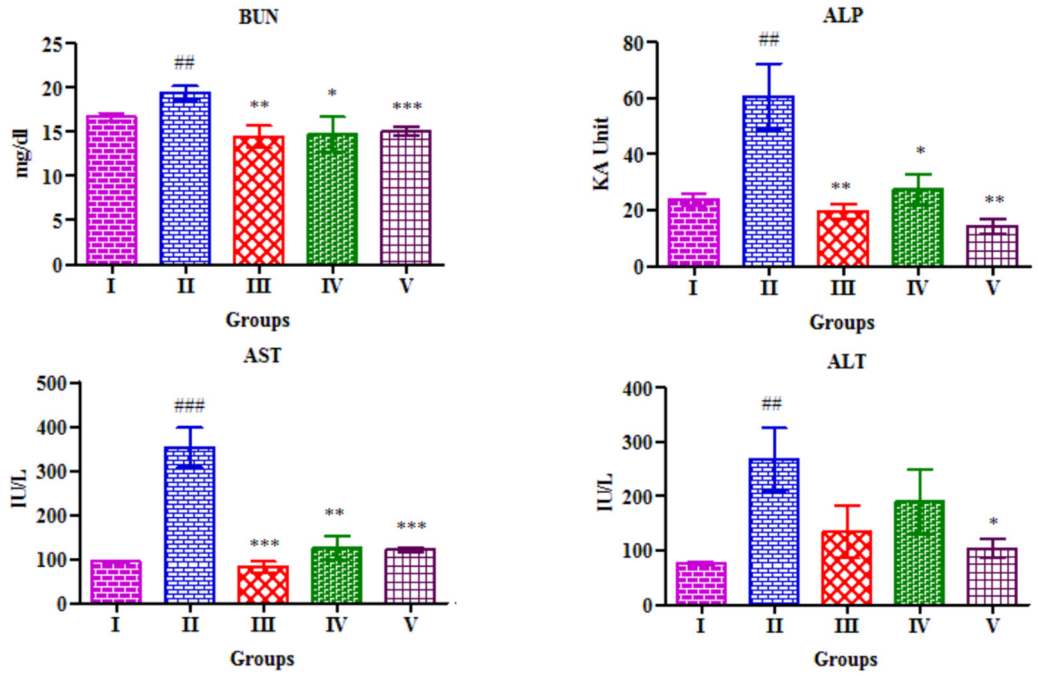
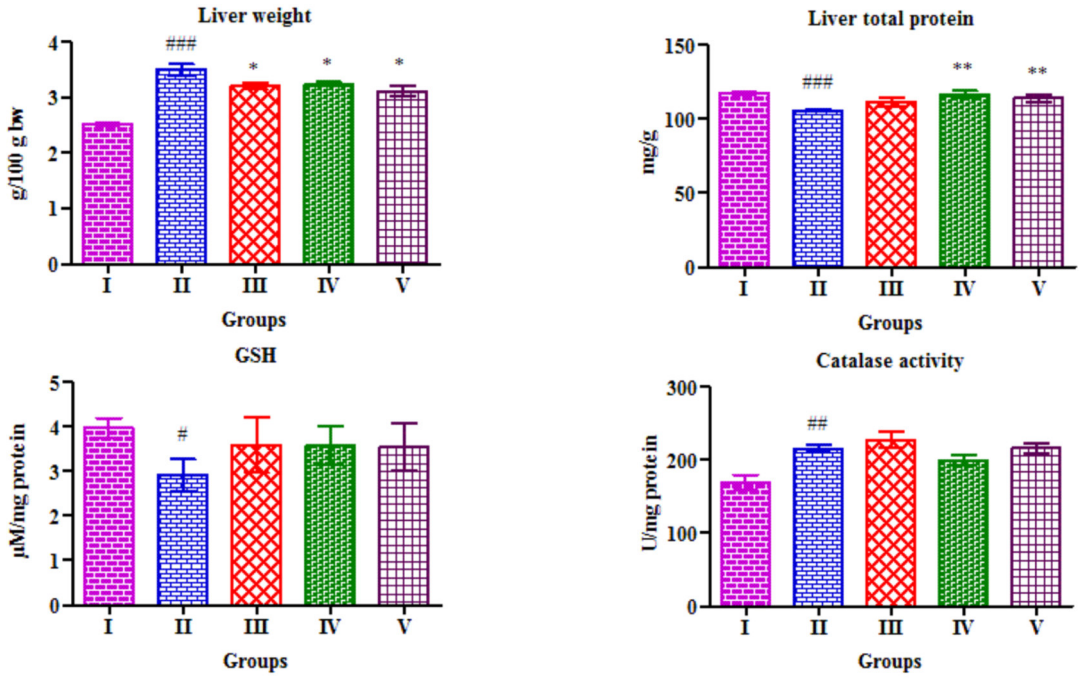


Figure 6: Effect of methanol extract of *Lilium candidum* flowers on different serum biochemical parameters in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: LCM-400 mg/kg + APAP, Group IV: LCM-600 mg/kg + APAP, Group V: Silymarin-100 mg/kg + 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.



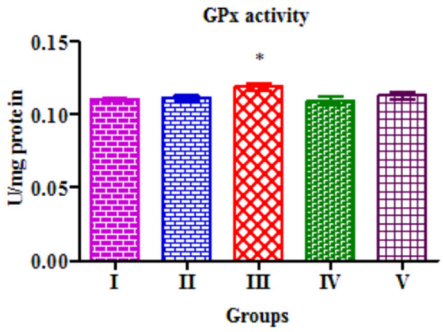


Figure 7: Effect of methanol extract of *Lilium candidum* flowers on relative liver weight, liver total protein and different liver antioxidants in APAP (3 g/kg) induced hepatic damage in rats. Group I: Normal control, Group II: Toxin control APAP, Group III: LCM- 400 mg/kg + APAP, Group IV: LCM-600 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.

4.7. 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.

DISCUSSION

PHYTOCHEMICAL ANALYSIS

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In phytochemical analysis, presence of alkaloids, phenolic compounds, tannins, flavonoids have been associated with various degrees of anti-inflammatory, analgesic and antioxidant activities. Therefore, the hepatoprotective effects observed in this study may be due to the activity(s) of one or a combination of some of the classes of compounds present in *Lilium candidum* flowers.

5.6. HEPATOPROTECTIVE STUDIES

Hepatic fibrosis is usually initiated by hepatocyte damage. Biologic factors such as hepatitis virus, bile duct obstruction, cholesterol overload, etc. or chemical factors such as CCl_4 administration, alcohol intake are known to contribute to liver fibrosis. The incidence of chronic fibrosis is high, but there are no satisfactory agents with ascertained effectiveness and with fewer side effects on liver. So, finding effective ways to inhibit liver fibrosis and prevent the development of cirrhosis are of great significance. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxic agent is the index of its protective effect.

5.6.1. Diclofenac induced hepatotoxicity

Hepatotoxicity is currently a class warning for NSAIDs and infrequent hepatic injury has been observed for nearly all NSAIDs currently on the market. There are 3 drugs that have more commonly been associated with liver disease: diclofenac, sulindac, and aspirin.

Diclofenac undergoes similar hepatic metabolism both in rat and in humans. Major metabolic pathways are the hydroxylation in position 4 and 5 and to a much lesser extent the formation of 3′-hydroxy- (humans) and 4′, 5-dihydroxydiclofenac (rat and humans). Diclofenac and its metabolites undergo extensive conjugation with glucuronic acid and sulfate. The major constitutive P450 form involved in diclofenac hydroxylation in man is cytochrome P4502C9, the human orthologous form of rat 2C11. Diclofenac forms selective protein adducts in livers of treated mice. This is caused by a transacylation reaction of its glucuronide conjugate. This mechanism has been proposed to explain both the allergic and intrinsic hepatotoxicity of the drug.

Since unwanted side effects of diclofenac in man and other mammals was reported to occur particularly in the liver it was thought of interest to evaluate LCM for its hepatoprotetive property in diclofenac induced hepatic damage in rats. In the present study, the administration of diclofenac to rats decreased the total protein and albumin level and increased the BUN level significantly. The pretreatment of LCM at two different dose levels restored the level of protein, albumin and BUN towards normalization. Hepatocellular injury from metabolic inhibition, oxygen radical toxicity, immunologically mediated damage, or some other mechanism results in predominant elevations of aminotransferase and alkaline phosphatase. The ALT, AST and ALP levels were significantly elevated when rats were administered with diclofenac indicating hepatocellular damage. The increased levels of these enzymes were significantly decreased by pretreatment with LCM in dose dependent manner. This is the indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by diclofenac. The hepatoprotetive property of *Polyalthia longifolia*, *Curcuma longa*, *Glycyrrhiza glabra* and *Moringa oleifera* in diclofenac induced hepatic damage in rats was reported.

The significant increased liver weight of diclofenac exposed animals seems to be due to toxic potential of diclofenac. The significant increase in weight of liver was, however, found to be associated with concomitant increase of serum AST and ALT enzyme levels. It is important to note that the elevated activity of serum AST and ALT recorded in this study may be due to loss of enzymes of liver tissue. Pretreatment of LCM decreased the liver weight significantly indicating recovery of liver tissue from damage. Significant decrease in total protein of the liver contents is a reflection of hepatic toxicity. The significant reductions of protein in diclofenac intoxicated group indicate depletion in the protein reserve and thus suggest hepatic toxicity. LCM administration increased the total protein content leading to normalization. GSH is an extremely efficient intracellular buffer for oxidative stress and GSH acts as a non-enzymatic antioxidant that reduces H_2O_2, hydroperoxides (ROOH) and xenobiotic toxicity.

The level of GSH depleted when animals were injected with diclofenac. The depleted level of GSH raised with the pretreatment of LCM. The catalase and GPx are enzymatic antioxidants widely distributed in all animal tissues that decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals.

Therefore, the reduction in the activity of these two enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present study, LCM significantly restored the hepatic catalase and GPx activity, which indicated that LCM could scavenge reactive free radicals that eventually lessen the oxidative damage to the tissues and subsequently improved the activities of these antioxidant enzymes.

Lilium candidum extract pretreatment prevented the reduction in the antioxidant enzyme activities and consequent oxidative damage to the liver. In fact, the multiple dose pretreatment of *L.candidum* extract alone significantly boosted the antioxidant enzyme activities**.** Molina et al. (2003), Srivastava and Shivanandappa (2010) also reported good hepatoprotective activity in their studies; and they suggested that the hepatoprotective activity of plant extract could be a result of boosting the antioxidant capacity of the liver.

5.6.2. Carbon tetrachloride induced hepatotoxicity

CCl_4 is a well-known hepatotoxic agent and the preventive action of liver damage by CCl_4 has been widely used as an indicator of liver protective activity of drugs in general. Hepatotoxicity induced by CCl_4 is the most commonly used model system for the screening of hepatoprotective activity of plant extracts/drugs. The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. Toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structure. CCl_4 is a xenobiotic that produces hepatotoxicity in various experimental animals.

CCl_4 is metabolized by cytochrome P450 to form a reactive trichloromethyl radical (CCl_3) and a trichloromethyl peroxyl radical (CCl_3O_2). Both radicals are capable of binding to DNA, lipids, proteins or carbohydrates, leading to lipid peroxidation, cell necrosis, and excessive deposition of collagen in liver and liver fibrosis. The effect of CCl_4 is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl_4 intoxication. The total protein and albumin levels decreased due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides. In the present study, CCl_4 intoxication reduced the serum total protein and albumin levels. The pretreatment of LCM restored the total protein and albumin levels. The rise in protein and albumin level suggests the stabilization of endoplasmic reticulum leading to protein

synthesis. The liver marker enzymes (AST, ALT and ALP) are cytoplasmic in nature; upon liver these enzymes enter into the circulatory system due to altered permeability of membrane.

In this study, significant increase in AST and ALT levels in the serum was observed after administration of CCl_4. ALP level also increased after CCl_4 administration. The increased levels of these enzymes significantly decreased by pretreatment with LCM extract. Reduction in the levels of AST, ALT and ALP towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4 .

Many studies have demonstrated that the hepatoprotective effect of plant extracts may be related to its antioxidant capacity to scavenge reactive oxygen species. CCl_4 intoxication reduced the total protein level in liver homogenate, which restored significantly with the pretreatment of LCM. Liver cells possess antioxidant defense system consisting of antioxidants such as GSH and antioxidant enzymes such as catalase and GPx to protect own cells against oxidative stress, which causes destruction of cell components and cell death. GSH is widely distributed among living cells and is involved in many biological functions, acting as an essential intracellular reducing agent for maintenance of intracellular redox status. It is also the most important biomolecule protecting against chemically induced cytotoxicity, by participating in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or by direct quenching of free radicals. CCl_4 intoxication slightly reduced the level of GSH, which was significantly restored in LCM treated (higher dose) rats. Trichloromethyl peroxy radical, the metabolic product of CCl_4 binds covalently to the macromolecules and causes peroxidative degradation of cellular membrane leading to the necrosis of hepatocytes. The hepatic antioxidant enzymatic activity of catalase and GPx significantly decreased in CCl_4- intoxicated rats as compared with control rats. The decreased enzymatic activity would result in an increased steady-state level of oxidants, contributing to cell injury.

The catalase level was elevated by administration of LCM to CCl_4 intoxicated rats suggesting that it has the ability to restore the enzyme activity towards normalization in CCl_4 damaged liver. However, administration of LCM to CCl_4 intoxicated rats had no effect in hepatic GPx activity and relative liver weight as compared to the CCl_4 treated toxin control group. This result suggests that LCM markedly inhibited CCl_4 induced liver damage by elevated hepatic antioxidant enzymatic system such as catalase and GSH. The rise in marker enzymes level in CCl_4 treated animals has been attributed to damaged structural integrity of the liver. Administration of the LCM preserved the structural integrity of the hepatocellular membrane as evidenced from attenuation of the marker enzymes level when compared to CCl_4 treated animals.

5.6.3. Acetaminophen induced hepatotoxicity

Acetaminophen (APAP), a frequently used analgesic and antipyretic drug, is known to be hepatotoxic in higher doses, which is primarily metabolized by sulfation and glucuronidation to unreactive metabolites, and then activated by the cytochrome P450 system to produce liver injury. It is established that acetaminophen is bioactivated to a toxic electrophile, N-acetyl p- benzoquinone imine (NAPQI), which binds covalently to tissue macromolecules, and probably also oxidizes lipids, or the critical sulphydryl groups (protein thiols) and alters the homeostasis of calcium. The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes. The experimental evidence suggests that during metabolism of this type of drug, different reactive metabolites are produced that covalently modify proteins, impose oxidative stress and causes mitochondrial injury.

In the present study, a reduction in total serum protein including albumin levels observed in the APAP treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein. But the treatment with LCM did not have protective effects in regards to total protein and albumin levels. Blood urea nitrogen (BUN) is also a marker of liver and renal functions, which is used to diagnose acute and chronic diseases related to liver and kidney. APAP administration to the rats increased the BUN level. The increase in BUN after APAP administration was prevented by LCM. The hepatic cells consist of higher concentrations of AST, ALT and ALP in cytoplasm and AST in particular exists in mitochondria. Due to the damage caused to hepatic cells, the leakage of plasma cause an increased level of hepatospecific enzymes in serum. The elevated serum enzyme levels are indicative of cellular leakage and functional integrity of cell membrane in liver.

The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been induced by a hepatotoxin. The measurement of serum AST, ALT and ALP levels serve as a means for the indirect assessment of condition of liver. The level of these enzymes significantly increased in serum, when animals were administered with APAP. The pre-treatment of the animals with LCM with respect to intoxication with APAP controlled the AST, ALT and ALP levels when compared with the toxic group.

The relative liver weight significantly increased in APAP intoxicated animals indicating toxic effect of APAP. Pretreatment of LCM decreased the liver weight significantly indicating recovery of liver tissue from

damage. GSH, a major known protein thiol in living organisms plays a central role in coordinating the body's antioxidant defense process. Excessive peroxidation causes increased GSH consumption. GSH is a scavenger of toxic metabolites, including NAPQI, which is a metabolite of APAP. GSH plays a major protective role as a scavenger of free radicals that combine with nonprotein thiols at the GSH reactive center to abolish free radical toxicity. Anti-oxidation by GSH protects the body from many diseases and conditions such as damage by H_2O_2, ethanol and numerous other toxins. Because GSH plays an important role in the antioxidant defense system, it becomes the key determinant in the APAP-induced hepatotoxicity. In the present study, the contents of liver protein and GSH in the APAP group decreased significantly after APAP administration, when compared with the control group. Pretreatment with LCM restored the total protein and GSH levels towards normalization. Administration of APAP as well as LCM did not have any effects in catalase and GPx activity.

5.7. ACUTE TOXICITY STUDY

Toxicology is a science to study adverse-effects of chemicals or physical agents on biological system and preclinical toxicology is a science to evaluate safety of drug (mostly) in animals to decide if the drug is safe for human use or not. Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially regarding their toxic aspects.

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide information relevant to acute overdosing in humans. It could also be used to estimate the therapeutic index (LD50/ED50) of drugs.

In the present study, acute toxicity test was done to establish if any adverse effects of the administration of the methanol extract of *Lilium candidum* on some observable and hematological parameters. The results indicate no abnormal symptoms and no death of the rats. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals.

In the present study, no significant changes were observed in the general behavior, body weight, feed and water intake of rats in the treated groups as compared to the control group, suggesting that at single oral doses administered, methanol extract of *Lilium candidum* flowers had no effect on the normal growth of rats. Organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs and in toxicological experiments, comparison of organ weights between control and treated groups have conventionally been used to predict toxic effect of a test material. In acute toxicity study in male rats at lower dose kidney weight increased while in female rats at higher dose kidney weight decreased significantly. The weight of lung in acute toxicity study increased in both male and female rats; but this was not associated with morphological changes and no evidence of toxicity was found. Increased testis weight and decreased uterus weight in treatment groups cannot be considered as a manifestation of toxicity due to the variability attributable to its small size and physiological factors unrelated to treatment like estrus cycle and relative infrequency of these organs as target organs of toxicity. The absence of significant changes in other organs in the present study points to the fact that ingestion of *Lilium candidum* methanol extract did not induce any anomalous growth or inflammation to these organs which would otherwise have resulted in higher relative organ weights in the treatment groups.

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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