Hepatoprotective Activity of Isolated Flavonoids of *Ficus glomerata* in Carbon Tetrachloride Induced Hepatotoxicity in Rats

Prashant Kalambe*, Hemant Sharma

Faculty of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Sehore, (M.P.), India

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Corresponding Author:

E-mail:
prashantkalambe1210@gmail.com
Mob.: +919039370985

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Abstract

The present examination was planned to isolate flavonoids from *Ficus glomerata* leaves separates, and surveyed their hepatoprotective action against CCl₄ instigated liver toxicity in rodents. The petroleum ether, ethanol and aqueous extracts were prepared and screened for phytochemical examination. The in vitro antioxidant activity namely DPPH, Superoxide Scavenging, total polyphenol content and total flavonol content activity of ethanol and aqueous extract were investigated. The distinctive portions were detached from ethanol extracts by utilizing column chromatography. The fractions F5, F7 and F8 were screened for hepatoprotective activity. The phytochemical study demonstrated the flavonoids and polyphenol components present in ethanol and aqueous extract. The findings of in vitro antioxidant activity suggested the ethanol extract have higher quantity of polyphenol and flavonoid component compared to aqueous extract. The fractions F5, F7 and F8 shows prominent hepatoprotective activity by altogether diminishing the CCl₄ initiated modification in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. The isolated compound altered the SOD, GPx, CAT and lipid peroxidation level, it indicates the antioxidant activity of isolated components. The findings of study recommend that the flavonoid compound F7 of *Ficus glomerata* showed higher hepatoprotective activity compared F5 and F8.

1 Introduction

Medicinal herbs and their inferred medicines are generally utilized in traditional culture systems throughout the world. In most recent couple of decades, there has been an exponential development in the field of herbal medicine and getting advanced in developing and developed countries inferable from its normal cause and lesser sick impacts. It is reported that 80% of the word populace has confidence in traditional medicine, especially plant drugs for their essential wellbeing care¹. Plants utilized for conventional medicine systems contain a wide range of substances that can be utilized to treat chronic, metabolic as well as infectious diseases. Recent trend, however, shows that the discovery rate of active chemical entities is declining. Natural products of higher plants may give a new source of antimicrobial and immunomodulatory agents with possibly novel mechanisms of action.

Ficus glomerata (Moraceae family) is commonly known as Gular, Umar, Umber, and Udumbara. It is a herb found all over India. It is an evergreen tree; common throughout the State near villages and along streams and rivers; also planted along road-side. Phytochemical screening on *Ficus glomerata* have announced the nearness of cycloartenol, euphorbol, hexacosanate, triacetate, taraxerone, tetratriterpene, glauanolacetate, racemosic acid, glauanol, glucose, hentriacontane. Plant is commonly used in antihyperlipidemic, diabetic, hepatoprotective, analgesic and wound healing^{2,3}.

The major challenges for researchers are to find out new potent medicines with fewer side effects, self-administrable, less expensive and completely reversible. The synthetic drugs is fail to fulfill these challenges. Further the herbal medicines could be used by minimizing above contests. Till date no potent synthetic drugs are available which use for the healing of hepatic

necrosis. The hepatoprotective activity of *Ficus glomerata* leaves extract was scientifically reported. However, no report has yet been published about the active component responsible for hepatoprotective activity of *Ficus glomerata* leaves extract. The main aim of this study was to evaluate the antioxidant and hepatoprotective activity of isolated compounds *Ficus glomerata* extracts.

2 Material and Method

2.1 Collection and identification of plant material

Ficus glomerata of voucher herbarium specimen were prepared and preserved along with crude drug sample at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, SSSUTMS, Sehore (M.P.), India. The plant materials were shade dried, reduced to coarse powder and stored in airtight container till further use.

2.2 Preparation of extract

The dried and powdered barks (250 gm) were successively extracted on a Soxhlet apparatus, employing petroleum ether, ethanol and distilled water respectively. The extracts were further concentrated under reduced pressure with a rotary evaporator.

2.3 Qualitative chemical tests

Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids⁴⁻⁶.

2.4 *In vitro* antioxidant activity of extract

2.4.1 Hydrogen-Donating Activity

In this experiment methanolic solution of DPPH (100 mM, 2.95 ml), 0.05 ml of each extracts dissolved in methanol was added at different concentrations (50-250 µg/ml). Reaction mixture was shaken and after 30 min at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity (% AA). Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract, was calculated by the following equation^{7, 8}

$$\% \text{ AA} = 100 - \{[(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100] / \text{Abs}_{\text{DPPH}}\}$$

2.4.2 Superoxide Scavenging Activity

Superoxide scavenging was carried out by using alkaline Dimethyl sulfoxide (DMSO). Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 ml) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phosphate buffer (10 mM, pH 7.4). Sample extract (1 ml) at various concentrations (50-250 µg/ml) in water was added and the absorbance was recorded at 560 nm against a control in

which pure DMSO has been added instead of alkaline DMSO⁸⁻¹⁰.

2.4.3 Total polyphenol content

Total polyphenol content was determined using colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin-Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve⁹⁻¹¹. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).

2.4.4 Total flavonol content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve^{11,12}. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

2.5 Isolation of Compounds from *Ficus glomerata* leaves extract

The *Ficus glomerata* extract was subjected to column chromatography using silica gel (60-120 mesh size), and eluted with the following solvent ratios of Hexane: Ethyl acetate (EA), 100:0, 80:20, 60:40, 40:60, 80:20, 0:100, then with 100:0, 80:20, 60:40, 40:60, 80:20, 0:100 for EA: Ethanol (Eth). The Eth:Methanol (MeOH) 100:0, 80:20, 60:40, 40:60, 80:20, 0:100. The fractions (25 ml) were collected from the column. The elute collected were monitored by thin layer chromatography (eluent: EA-Eth, 9:1 and 3:2) for homogeneity and the similar fraction were pooled together. The eleven different fractions were collected and dried. The fraction F1, F2 and F3 were containing waxy material; the fractions F4, F6, F9 and F11 were powder but quantity was very little. The yield of fraction F5, F7, F8 and F10 were 340 mg, 430 mg, 365 mg and 210 mg, respectively (Table 6.14). The four fractions were further analyzed for phytochemical screening to determine the nature of isolated compound.

2.6 Hepatoprotective activity

2.6.1 Selection of animals

Male Wistar rats (150-200 gm), were used, and kept in quarantine for 10 days under standard husbandry conditions (27.3 °C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad libitum*. All experiments were approved by the institutional ethical committee and were carried out according to the animal ethics committee guidelines.

2.6.2 CCl₄-induced hepatotoxicity

Groups II to VI received CCl₄ (1ml /kg i.p.) with equal volume of olive oil (50% v/v) to induce acute toxicity, for two successive days. Group II animals were maintained as CCl₄ group, while Groups III to V animals were treated orally for seven days with F5, F7 and F8 at the dose of 50 mg/kg, respectively. Group VI animals were treated with standard drug silymarin (25 mg/kg). After the drug treatment all the animals were sacrificed by cervical dislocation. Blood was collected from the carotid artery and was allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters.

Serum separated by centrifugation were used to determined serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and serum bilirubin¹³⁻¹⁵.

2.6.3 Liver Homogenates Preparation and Biochemical Estimation

Frozen liver samples were homogenized (with a Potter-Elvehjem homogenizer) in a Tris-HCl buffer or in a phosphate buffer solution (PBS) to give a 20% homogenate. To evaluate levels of lipid peroxidation, the homogenate was centrifuged at 1700 rpm/min for 10 min at 4 °C. To assess catalase activity, the homogenate was centrifuged at 3500 rpm/min for 15 min at 4 °C and then diluted up to 5%. Further on, the supernatant was again centrifuged either at 10,000 rpm/min for 1 min and diluted to 2% for measurement of glutathione peroxidase activity or at 30,000 rpm/min for 10 min before extraction of tissue superoxide dismutase activity with 20% ethanol¹⁶⁻²¹.

2.7 Statistical analysis

The results are expressed as mean $\pm$ SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A P < 0.05 value was considered as statistically significant.

3 Results and Discussions

3.1 Phytochemical screening of *Ficus glomerata*

Preliminary phytochemical investigations of the extracts of leaves of *Ficus glomerata* revealed the presence of flavonoids, tannins, phenolic compounds, alkaloids, glycosides, fats and carbohydrates. The details are presented in table 1.

From the result of phytochemical screening, the petroleum ether extract of leaves of *Ficus glomerata* exhibited the presence of steroids, fats and oils. Alkaloids, glycosides, carbohydrates, flavonoids, tannins, proteins and polyphenol were found in ethanol extracts of leaves of *Ficus glomerata*. Similarly glycosides, carbohydrates, flavonoids, tannins and polyphenol

were existing in aqueous extracts of leaves of *Ficus glomerata*. The maximum phytocostituents were observed in ethanol extracts of leaves of *Ficus glomerata* (Table 1). Now ethanol and aqueous extracts of *Ficus glomerata* was selected for further *in vitro* antioxidant activity evaluation as this extract revealed the presence of flavonoids and phenolic compounds.

Table 1: Phytochemicals present in leaves of *Ficus glomerata* extracts

Phytoconstituent	Pet. Ether	Ethanol	Aqueous
Alkaloids	-	+	-
Glycosides	-	+	+
Carbohydrates	-	+	+
Tannins and Phenolic compound	-	+	+
Flavonoids	-	+	+
Steroid test	+	-	-
Protein	-	+	-
Fat and oil test	+	-	-

+ = Present, - = Absent

3.2 *In vitro* antioxidant activity

The ethanol and aqueous extract of *Ficus glomerata* was subjected to *in vitro* antioxidant studies to determine and compare the antioxidant activities of extracts. Antioxidant ability of *Ficus glomerata* extract was assessed by establishing its efficacy in hydrogen-donating, total polyphenol content and total flavonol content.

3.2.1 Hydrogen-Donating Activity of *Ficus glomerata*

As shown in Fig 1 and 2, *Ficus glomerata* of ethanol and aqueous extracts strongly scavenged DPPH radical with the IC₅₀ being 62.91 and 92.57 μ g/ml, respectively. The scavenging was found to dose dependent. The standard drug ascorbic acid strongly scavenged DPPH radical with the IC₅₀ being 47.74 μ g/ml (Fig 3).

Fig. 1: IC₅₀ values of ethanol extracts of *Ficus glomerata*

Fig. 2: IC₅₀ values of aqueous extracts of *Ficus glomerata*

Fig. 3: IC₅₀ values of ascorbic acid

3.2.2 Superoxide scavenging assay

The IC₅₀ value for ethanol and aqueous extracts of *Ficus glomerata* were 72.05 and 93.59 µg/ml, respectively (Fig. 4 and Fig. 5). The superoxide radical scavenging activity was found to dose dependent. The standard drug ascorbic acid strongly scavenged superoxide radical with the IC₅₀ being 55.77 µg/ml (Fig 6).

Fig 4: IC₅₀ values of ethanol extract of *Ficus glomerata* by superoxide scavenging radical

From results, it was found that the extracts showed moderately to strongly free radical scavenging activity. The extracts donated their electrons to the superoxide and scavenge them to

prevent their further interaction with NBT followed by inhibition of formation of blue colored formazan product^{22,23}. The outcomes of results revealed that the ethanol extracts displayed high content of flavonoids, which was significantly correlated with the superoxide radical scavenging activity.

Fig 5: IC₅₀ values of aqueous extract of *Ficus glomerata* by superoxide scavenging radical

Fig 6: IC₅₀ values of ascorbic acid by superoxide scavenging radical

From the result of DPPH and superoxide radical scavenging activity it was observed that the ethanol extracts of *Ficus glomerata* showed highest DPPH and superoxide radical scavenging activity. It indicates the presence of antioxidant components in crude extracts of *Ficus glomerata*. From these results it can be concluded that antioxidant activity of extracts depends on the presence of quality of active constituents, because each *in vitro* antioxidant model has different mechanism to reduce free radicals.

Earlier many researchers have reported that the antioxidant activity of extracts is directly proportional to the phenolic and flavonol contents. Following it as a guidelines total flavonol and polyphenol content was determined in ethanol and aqueous extracts.

3.2.3 Total phenolic content of *Ficus glomerata*

Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data (Fig 7). The linear

equation of gallic acid was found to be $y = 0.0514x - 0.006$ (Fig 7). The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in table 2.

Fig 7: Calibration curve of gallic acid in distilled water

Table 2: Determination of total polyphenol content of *Ficus glomerata*

Extract	Total polyphenol content (GAE mg/gm)
Ethanol	78.13±1.07
Aqueous	52.42±1.47

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

The total phenolic content in extracts, expressed as gallic acid equivalents. The total phenolic content of ethanol and aqueous extract of *Ficus glomerata* were 78.13 and 52.42 GAE mg/gm, respectively. The ethanol extracts exhibited highest amount of total polyphenol content compared to aqueous extracts.

3.2.4 Total flavonol content of *Ficus glomerata*

Standard curve of quercetin was calculated and plotted in distilled water for determining absorption data (Fig 8). The linear equation of quercetin was found to be $y = 0.036x - 0.0032$ (Fig 8).

Fig 8: Calibration curve of quercetin in distilled water

The content of flavonoids identified in the tested extracts is shown in table 3. The concentrations of flavonoids in ethanol and aqueous extract of *Ficus glomerata* were 67.52 and 51.29 QE mg/gm, respectively. The ethanol extracts exhibited highest amount of flavonoids content compared to aqueous extracts.

Table 3: Determination of total flavonol content of *Ficus glomerata*

Extract	Total flavonol content (GAE mg/gm)
Ethanol	67.53±0.72
Aqueous	51.29±0.53

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

The plant flavonoids and polyphenols are greatly effective free radical scavenging and antioxidants activity²⁴. The diseases associated with free radicals are healed by polyphenol and flavonoids. The phenolic compounds have been recognized as antioxidant and have been known to show medicinal activity as well as for exhibiting physiological functions²⁵⁻²⁹. The hydroxyl group present in flavonoids is responsible for radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes. It is well known that plant phenolics, in general are highly effective in free radicals scavenging, and they are antioxidants.

The findings of total polyphenol and flavonol content of ethanol and aqueous extract of *Ficus glomerata* supports the study of DPPH and superoxide scavenging capacity of extracts.

From the results of antioxidant, it can be concluded that ethanol extracts of *Ficus glomerata* produces higher antioxidant activity compared to aqueous extract and could alleviate the number of oxidative stress induced hepatotoxicity. The above study was done only for proper selection of extracts from *Ficus glomerata*, to isolate the active constituent from extract expressing maximum antioxidant activity. Hence the ethanol extract of *Ficus glomerata* was selected for the isolation of active constituents by column chromatography.

3.3 Isolation of compound from ethanol extract of *Ficus glomerata*

The four fractions namely F5, F7, F8 and F10 selected and further analyzed for phytochemical screening to determine the nature of isolated compound.

3.3.1 Preliminary phytochemical analysis of isolated fraction of ethanol extract of *Ficus glomerata*

The phytochemical investigation of F10 of *Ficus glomerata* revealed the presence of alkaloids and carbohydrates. The F7 demonstrated the presence of glycosides, flavonoids, tannins & phenolic compounds. The F5 and F8 indicate the presence of tannins & phenolic compounds and flavonoids (Table 4).

The secondary metabolites impart various pharmacological activities namely anti-tumor, analgesic, anti-arthritis, antidiabetic, hepatoprotective etc. The fractions obtained from the ethanol extract of *Ficus glomerata* exhibited various types of secondary metabolites. Many investigations have proven that varieties of flavonoid molecules possess hepatoprotective activity. Thus, it may be valuable to continuously evaluate the hepatoprotective activity of flavonoids, not only for establishing hepatoprotective mechanisms, but also for developing a new class of hepatoprotective agents. The F5, F7 and F8 containing polyphenol and flavonoids compound and these organic substances impart chief role in hepatoprotective activity. Hence this result supports us to evaluate the hepatoprotective activity of the F5, F7 and F8.

Table 4: Preliminary phytochemical analysis of isolated fraction of ethanol extract of *Ficus glomerata*

Phytoconstituents	F5	F7	F8	F10
Alkaloids	-	-	-	+
Glycosides	-	+	-	-
Carbohydrates	-	-	-	+
Tannins & Phenolic compounds	+	+	+	-
Flavonoids	+	+	+	-
Steroids	-	-	-	-
Proteins and Amino acids	-	-	-	-
Fixed Oils and Fats	-	-	-	-

(+) Present, (-) Absent

3.4 Hepatoprotective activity

The *in vitro* studies of the F5, F7 and F8 isolated from *Ficus glomerata* leaves extracts indicates the presence of flavonoids and polyphenol. Therefore hepatoprotective activity were performed with F5, F7 and F8.

3.4.1 CCl₄ induced hepatotoxicity

Hepatoprotective activity of chloroform and ethanol extracts were determined in CCl₄ induced hepatotoxicity model. In the present studies, rats treated with CCl₄, developed significant liver damage as observed from the elevated serum levels of hepato-specific enzymes as well as severe alteration in other biochemical parameters. Values of the biochemical parameters were significantly increased in the CCl₄ treated rats (Table 5).

Treatment with both doses of F8 and F9 isolated from of *Ficus glomerata* decreased the CCl₄ induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. It was found that the test samples offer protection against toxin as evidenced by remarkable reduction in all biochemical parameters (P<0.05). It also revealed that decreased

biochemical parameter levels of both isolated compound are less compared to standard drug. The fraction F7 exhibited maximum hepatoprotective activity compared with F5 and F8.

3.4.2 In-vivo antioxidant activity

Results from the antioxidant evaluation are shown in table 6. The administration of the F8 and F9 isolated from of *Ficus glomerata* counteracted the CCl₄-induced free radical activity, which resembles that of silymarin. SOD, GPx and CAT enzyme levels are statistically significant increased, whereas lipid peroxidation is decreased, when compared to CCl₄ condition (P < 0.05). These results suggest that the F5, F7 and F8 isolated from of *Ficus glomerata* displays an antioxidant activity. The fraction F7 exhibited maximum antioxidant activity compared to F5 and F8.

Liver participate in several metabolic activities, and in order to fulfill this role, release a wide variety of enzymes. Liver can be injured by many toxicants, as well as by chemicals or drugs. In our model, CCl₄ serves as a toxicant. CCl₄-related hepatotoxicity is associated with elevation in enzyme levels, which may be attributed to the generation of trichloromethyl free radical during metabolism by the hepatic microsomes, which in turn begin lipid peroxidation.

Hepatocellular necrosis decreases SOD, CAT and GPx activities, and the increase of such activities into basal values, is a clear indication of plasma membrane stabilization and tissue repair as well. Such an effect is in agreement with the view that enzyme activities are restored into normal conditions and healing of the hepatic parenchyma, as well as hepatocyte regeneration, are observed. SOD, CAT, and GPx constitute an enzyme defense mechanism against oxidative damage.

Under CCl₄ conditions such enzyme activities are decreased, but under plant-treated conditions, a significant increase in their activities is observed, which may serve as a biochemical strategy to reduce lipid peroxidation^{30,31}. The study revealed that the F8 and F9 isolated from of *Ficus glomerata* under evaluation, at both studies doses, showed a hepatoprotective activity against CCl₄-induced liver damage. The hepatoprotective activity of F8 and F9 isolated from *Ficus glomerata* suggested their antioxidant activity due to polyphenol and flavonoid nature.

4 Conclusion

The F5, F7 and F8 isolated from of *Ficus glomerata* exhibited strong antioxidant activity and may confer a beneficial effect against oxidative stress. Based on the results of *in vivo* assay, the hepatoprotective activity of F5, F7 and F8 isolated from *Ficus glomerata* was due to its antioxidant property. From findings it has been concluded that the F7 of *Ficus glomerata* exhibited maximum pharmacological activities compared F5 and F8.

Table 5: Effect of isolated flavonoid component of *Ficus glomerata* on SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in CCl₄ induced liver toxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct (mg/dl)	Total (mg/dl)
Normal Control CMC (10ml/kg)	73.5±3.2	134.6±5.5	176.4±3.2	141.1±2.6	0.39±0.5	0.61±0.1
CCl ₄ (1ml/kg i.p.)	265.4 ^a ±5.1	352.7 ^a ±4.6	338.4 ^a ±3.1	349.7 ^a ±4.6	3.65 ^a ±0.3	5.39 ^a ±0.5
F5 (50 mg/kg)	98.8*±4.2	152.2*±3.2	195.8*±4.2	159.2*±2.9	0.52*±0.7	0.89*±0.8
F7 (50 mg/kg)	75.3*±5.6	126.7*±4.9	172.5*±4.5	139.3*±5.3	0.35*±0.8	0.62*±0.7
F8 (50 mg/kg)	85.8*±3.9	136.1*±3.6	183.9*±3.2	145.2*±2.4	0.49*±0.5	0.82*±0.8
Silymarin (25 mg/kg oral)	71.1*±4.2	129.6*±4.5	168.4*±5.8	139.7*±3.2	0.32*±0.5	0.56*±0.7

Values are expressed as mean ± SEM, n = 6 in each group. ^aP<0.05 when compared with normal control group, *P<0.05 when compared with CCl₄ treated group.

Table 6: Antioxidant activity of isolated flavonoid component of *Ficus glomerata* in CCl₄ induced liver damage in rats

Treatment	Catalase (mg liver protein) ⁻¹	Superoxide dismutase (mg liver protein) ⁻¹	Glutathione Peroxide (mg liver protein) ⁻¹	TBA (mg/liver protein)
Normal Control CMC (10ml/kg)	243.5±4.2	92.4±4.2	2.36±0.7	1.25±0.2
CCl ₄ (1ml/kg i.p.)	98.1±3.3 ^a	15.3±6.3 ^a	0.12±0.2 ^a	6.34±0.7 ^a
F5 (50 mg/kg)	196.1±5.5*	71.8±4.5	1.81±0.1	1.96±0.3*
F7 (100 mg/kg)	219.2±2.4*	81.7±2.9*	2.05±0.5*	1.53±0.6*
F8 (50 mg/kg)	201.6±3.8*	75.2±4.5*	1.86±0.3	1.72±0.1*
Silymarin (25 mg/kg)	235.9±4.2*	96.5±2.1*	2.52±0.4*	1.38±0.3*

Values are expressed as mean ± SEM, n = 6 in each group. ^aP<0.05 when compared with normal control group, *P<0.05 when compared with CCl₄ treated group.

5 Conflict of interest

Nil

6 Author's contribution

The PK and HS performed the experimental work and drafted the manuscript. Both authors approved the manuscript for publication.

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