Original Article

Evaluation Of Hepatoprotective Effect Of Dietary Inclusion Of Ficus Glomerata Fruits Against Cisplatin-Induced Hepatic Damage In Rats

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Abstract

In the present study, cisplatin-induced liver impairment was evidenced by an increase in serum ALT, ALP, and AST and hepatocyte cell degeneration, inflammatory infiltrate, and necrosis. These changes persisted on the 6th day due to a single dose of 6 mg/kg cisplatin. The 2% and 4% of *Ficus glomerata* normalized serum ALT, ALP, AST, LOD, GSH, SOD, and catalase of the liver. The researchers reported that cisplatin administration induced a significant increase in serum ALT, AST, and ALP and a significant decrease in serum total bilirubin, total protein, and albumin levels. The ability of cisplatin to cause alterations in the activity of these enzymes could be a secondary event following Cisplatin-induced liver damage with the consequent leakage from hepatocytes. The present study revealed that significantly decreased levels of ALT, ALP, and AST in blood serum due to their antioxidant effects and their ability to act as free radical scavengers, thereby protecting membrane permeability after treatment with 2% and 4% of *Ficus glomerata* indicate hepatoprotective and curative effects.

Actually, cisplatin contributed to various mechanisms of liver dysfunction consisting of cellular toxicity, vasoconstriction in the renal microvasculature, and proinflammatory effects, by producing free radical oxidative stress, which participated in the decline of antioxidant enzymes in the liver and led to liver injury. Hence, the possible mechanism of hepatoprotection and curative effect of *Ficus glomerata* supplement could be due to its good antioxidant potential content; it might contribute to free radical scavenging and antagonistic hepatotoxicity that were produced by cisplatin in liver injury.

Keywords: *Ficus glomerata*, Cisplatin, Necrosis, Bilirubin, Hepatoprotective

1. INTRODUCTION

Ever since the birth of mankind, there has been a relationship between life, disease, and plants. Primitive men started studying diseases and treatments. There is no record that people in prehistoric times used synthetic medicines for their ailments, but they tried to make use of the things they could easily procure [1]. The most common thing they could find was their environment, i.e., the plants and animals. They started using plants and found that the majority of plants were suitable as food, whereas others were either poisonous or medicinally useful. From their experience, this knowledge of herbal remedies was passed from generation to generation as folk medicine [2]. So the history of herbal medicine is as old as human history. Herbal medicine is still the mainstay of primary health care for about 75–80% of the world's population, mainly in developing countries, because of its better cultural acceptability, better compatibility with the human body, and fewer side effects. It is estimated that approximately [3] one quarter of all prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. Aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are a few important examples of what medicinal plants have given us in the past [4]. Most of these plant-derived drugs were originally discovered through the study of traditional cures and folk knowledge of indigenous people, and some of these could not be substituted despite the enormous advancement in synthetic chemistry. Consequently, plants can be described as a major source of medicine.

2. MATERIAL AND METHODS

All chemicals used were of analytical grade. Chemicals and reagents used for the preparation of buffers, analytical solutions, and other experimental purposes are listed in **Table 1.**

Table 1. Chemicals and reagents used in experimental purpose

S. No.	Chemical	Supplier/ Manufacturer
1	Petroleum ether AR	Qualigens Fine Chemicals, Mumbai
2	Methanol AR	Qualigens Fine Chemicals, Mumbai
3	Ethanol AR	Qualigens Fine Chemicals, Mumbai
4	potassium ferricyanide	Qualigens Fine Chemicals, Mumbai
9	Chloroform AR	Merck (P) Ltd, Mumbai

2.1 Collection and identification of plant material

Based on the information collected from the tribal people of Madhya Pradesh, the fruit part of the plant was selected for the proposed study. The plants were initially identified by their vernacular names through consultations with the local people. The plant materials were shade dried, reduced to coarse powder, and stored in an airtight container until further use.

2.1.1 Preparation of extract

Shade dried ficus glomerata fruit powder was macerated with water in a closed flask.

2.1.2 Extraction with water (Maceration)

The marc was dried in a hot air oven and macerated with water for 7 days with occasional shaking to get an aqueous extract. The aqueous extract was concentrated.

2.2 Qualitative chemical tests

2.2.1 Biuret test

The plant may be considered a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, protein, and lipids that are utilized as food by men, but also for a multitude of other compounds like glycosides, alkaloids, volatile oils, tannins, etc., that exert a physiologic effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents. Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins, and terpenoids.

2.2.2 Test for alkaloids

2.2.2.1 Dragendorff's test

To 1 ml of the extract, add 1 ml of dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

2.2.2.2 Mayer's test

To 1 ml of the extract, add 1 ml of mayer's reagent (potassium mercuric iodide solution). A whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

2.3 Test for Proteins

2.3.1 Ninhydrin test:

Add two drops of freshly prepared 0.2% Ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. The development of blue revealed the presence of proteins, peptides, or amino acids.

2.3.2 Millon's test:

1 mL of the test solution was made acidic with sulfuric acid, and then it was added to Millon's reagent and boiled. A yellow precipitate was formed, which indicated the presence of protein.

2.3.3 Xanthoproteic test;

Test solution was treated with concentrated nitric acid and boiled, which gives a yellow precipitate and confirms the presence of protein.

2.4 Test for Glycosides

2.4.1 Legal test:

Test solution was treated with a drop of 2% sodium nitroprusside and a drop of sodium hydroxide, resulting in the formation of a deep red color that showed the presence of glycoside.

2.4.2 Baljet test:

To 1ml of the test extract, add 1mL of sodium picrate solution, and the yellow to orange color reveals the presence of glycosides.

2.4.3 Keller-Killiani test:
The test solution was treated with a few drops of ferric chloride solution, mixed, and then sulphuric acid containing ferric chloride solution was added, forming two layers. The lower layer showed a reddish-brown color, and the upper layer turned bluish green, which showed the presence of glycosides.

2.5 Test for carbohydrates and sugars
2.5.1 Molisch's test:
To 2 ml of the extract, add 1 ml of alpha-naphthol solution. Add concentrated sulfuric acid through the side of the test tube. The purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

2.5.2 Fehling's test:
To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of reducing sugars

2.5.3 Benedict's test:
To 5 mL of Benedict's reagent, add 1 mL of extract solution and boil for 2 minutes, and cool. The formation of a red precipitate shows the presence of sugars.

2.6 Test for tannins and phenolic compounds
Take the small quantity of test solution and mix it with the basic lead acetate solution. The formation of white precipitates indicates the presence of tannins. To 1 mL of the extract, to a ferric chloride solution, the formation of a dark blue or greenish black product shows the presence of tannins. The small quantity of test extract is treated with potassium ferricyanide and ammonia solution. A deep red color indicates the presence of tannins.

2.7 Test for flavonoids
The extract is treated with sodium hydroxide, and the formation of a yellow color indicates the presence of flavones.
The extract is treated with concentrated H_2SO_4, formation of a yellow or orange color indicates flavones.

2.8 Test for steroids
2.8.1 Libermann-Burchard test:
1 gm of the test substance was dissolved in a few drops of chloroform, 3 ml of acetic anhydride, and 3 ml of glacial acetic acid were added, warmed and cooled under the tap, and drops of concentrated sulfuric acid were added along the sides of the test tube. The appearance of a bluish-green color shows the presence of sterols.

2.8.2 Salkowski test:
dissolve the extract in chloroform and add an equal volume of conc. H_2SO_4. The formation of a bluish red to cherry color in the chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.8.3 Spot Test:
Press a small quantity of extracts between the two pieces of filter paper. Oil stains on paper indicate the presence of fixed oils.

2.8.9 Saponification test:
Dilute 1 ml of extract with 20 ml of distilled water and shake in a graduated cylinder for 15 minutes. 1 cm of foam indicates the presence of saponins

3. EXPERIMENTAL PROTOCOL
1. Collection, identification and preparation of plant
2. Preparation of dietary inclusion
3. Evaluation of Chemoprotective activity of dietary inclusion against cisplatin induced hepatotoxicity
4. Histopathology investigation of isolated organ.

4. SELECTION OF ANIMALS AND PROCUREMENT
i. Male wistar rats were chosen for the study. (n =6)
ii. The animals were used after an acclimatization period of 7 days to the laboratory environment. They were provided with food and water *ad libitum*.

4.1 Preparation of Dietary inclusion
The basal diet (50% skimmed milk, 36% corn starch, 10% groundnut oil, and 4% mineral and vitamin premix) was prepared and fed to normal and control group animals. The basal diet was supplemented with 2% w/w and 4% w/w powdered fruit pulp of *Ficus glomerata,* respectively, and fed to normal and hepatotoxicity induced animals [8].

4.2 Experimental animals

The adult Wistar albino rats of either sex, weighing 150 - 180 g were used for the study. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husks as bedding. Animals were housed in a temperature of 24 ± 2 ºC and a relative humidity of 30-70%. A 12/12 h light and dark cycle was followed.

4.3 Hepatoprotective activity of dietary inclusion against Cisplatin induced hepatotoxicity

All the animals were divided into five groups; each group consisted of 6 animals, and they received the treatment as follows:

1. Group I: Normal and received basal diet
2. Group II: Received basal diet + 4% w/w *Ficus glomerata* fruit
3. Group III: Single administration of Cisplatin (6 mg/kg i.p.) + Control group received Basal diet for 7 days
4. Group IV: Single administration of Cisplatin (6 mg/kg i.p.) + 2% w/w *Ficus glomerata* for 7 days
5. Group V: Single administration of Cisplatin (6 mg/kg i.p.) + 4% w/w *Ficus glomerata* for 7 days

The protocols ended after 7 days. The animals were decapitated by cervical dislocation. The blood was withdrawn from animals by direct heart puncture and kept in bottles containing EDTA. The plasma was separated from the blood by centrifuges at 3000 r/min for 10 min. Furthermore, tlasma was used for the determination of various biochemical parameters. Correspondingly, the liver was isolated, rinsed in cold saline, and homogenized in phosphate buffer (pH 6.9). The clear supernatant was obtained after centrifuging the homogenates at 7500 r/min for 10 min. The *in vivo* antioxidant activity was estimated from the clear supernatant.

5. BIOCHEMICAL PARAMETERS

Serum separated by centrifugation was used to measure serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP), and total bilirubin [9, 10, 11, 12, 13].

6. ANALYSIS OF ANTIOXIDANT ENZYMES OF LIVER TISSUE

The antioxidant activities in the rat kidney homogenate were assayed for superoxide dismutase (SOD), Catalase (CAT) Glutathione (GSH), and activity lipid peroxidation (LPO). The activity of SOD was evaluated by the Nitro Blue tetrazolium (NBT) reduction process. The H_2O_2 technique was employed for the assessment of CAT activity. The substrate 5, 5'- Dithio.bis (2–nitrobenzoic acid) was used for the estimated GSH level. The lipid peroxidation level was estimated by measuring the concentrations of malondialdehyde [14, 15, 16].

7. HISTOPATHOLOGY OF LIVER

The liver was separated from the experimental animals of each group and washed with normal saline. The isolated organs were fixed at 10% buffered neutral formalin. After that, it was processed for paraffin implanting and followed the microtome technique for sectioning organs. The sections obtained were processed in the alcohol xylene series and were stained with alum, haematoxylin, and eosin. The stained sections were observed under a microscope for the evaluation of histopathological changes in liver.

8. DATA ANALYSIS

Results were analyzed using one way analysis of variance (ANOVA) followed by the tukey's test by using the statistical software package, GraphPad Prism; version 5.03. Values were expressed as mean ± SEM and $p < 0.05$ were considered statistically significant.

9. RESULTS AND DISCUSSION

9.1 Hepatoprotective activity against Cisplatin induced hepatotoxicity

It is well documented that plant flavonoids and phenols in general, are highly effective free radical scavenging antioxidants. The flavonoids present in the medicinal plants interrupt the production of free radicals and prevent cellular destruction inside the liver.

9.2 Hepatoprotective activity

Table 5.1 showed the effect of various treatments on serum SGOT, SGPT, ALP, ACP, and bilirubin. The animals treated with cisplatin developed significant liver damage, as observed from the alteration in the activities of serum enzymes (SGOT, SGPT, ALP, and ACP) and bilirubin in serum **(Table 2 and Fig. 2a-2e)**. The levels of the SGOT, SGPT, ALP, ACP, and bilirubin were significantly higher in the Cisplatin treated rats compared to normal animals fed a basal diet. The animals treated with diets containing 2% w/w and 4% w/w *Ficus glomerata* significantly reduced the serum level. The serum levels of animals receiving diets containing only 4% w/w *Ficus glomerata* (Group II) were normal, which indicates that liver functions were not affected.

Table 2. Effect of diets supplemented with *Ficus glomerata* on liver function test for different parameters in animals treated with Cisplatin

G Group No.	Treatment	SGOT (AST) (U/L)	SGPT (ALT) (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
						Direct (mg/dl)	Total (mg/dl)
I	Normal rats (Basal diets)	72.14 4.18	91.34 5.28	158.42 7.64	130.17 4.32	0.35 0.25	0.89 0.68
II	*Ficus glomerata* (4%) + Normal rats	73.62 2.95	95.51 3.72	157.28 5.19	129.37 6.25	0.39 0.64	0.85 1.32
III	Control rats (Cisplatin + Basal diets)	178.24 5.32*	194.56 4.86*	291.91 3.72*	246.64 4.34*	2.14 1.05*	3.72 1.24*
IV	*Ficus glomerata* (2%) + Cisplaatin(6 mg/kg)	118.35 4.83[a]	125.73 5.49[a]	198.31 7.54[a]	185.52 5.41[a]	0.98 1.13[a]	1.85 0.84[a]
V	*Ficus glomerata* (4%) + Cisplatin (6 mg/kg)	81.59 4.17[a]	94.72 5.64[a]	161.24 5.32[a]	135.65 4.22[a]	0.42 0.95[a]	0.85 0.49[a]

Values are expressed as mean SEM, n = 6 in each group. *P<0.05 when compared with normal group and normal *Ficus glomerata* (4%)**,** [a]P<0.05 when compared with Cisplatin treated group considered as statistically significant.

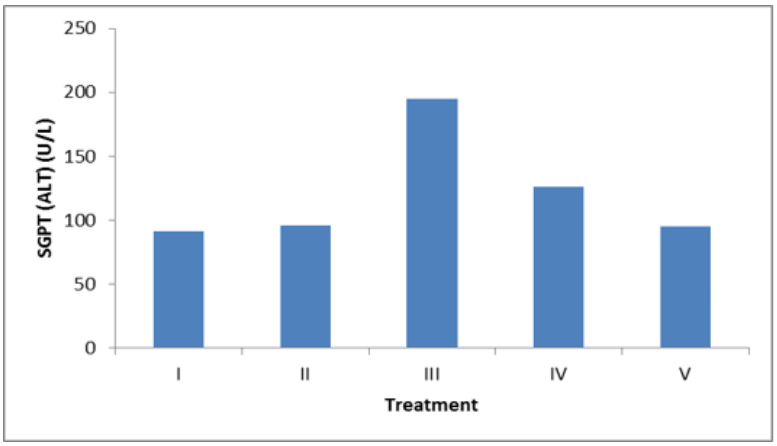

Fig 2a. Level of SGOT in various treatment groups

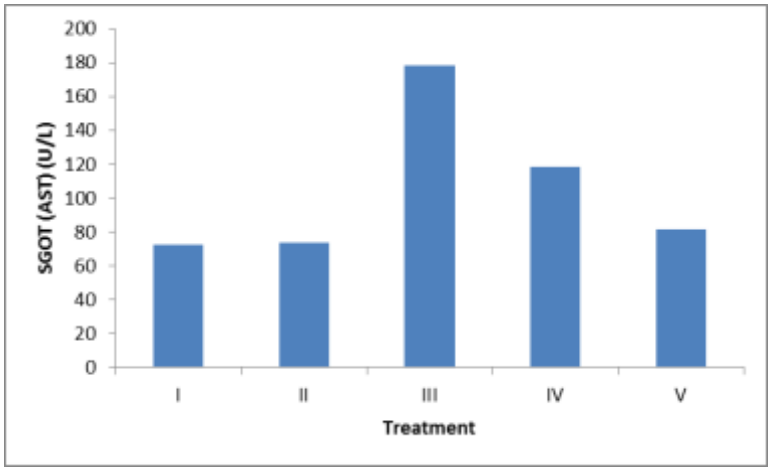

Fig 2b. Level of SGPT in various treatment group

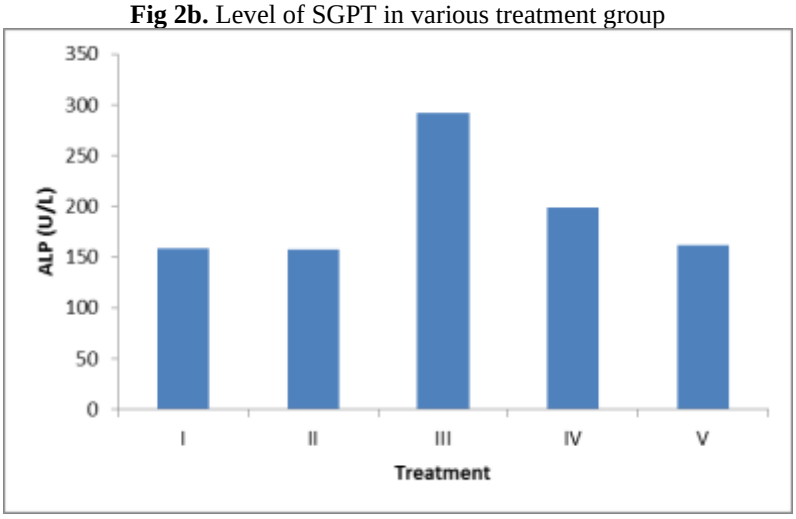

Fig 2c. Level of ALP in various treatment groups

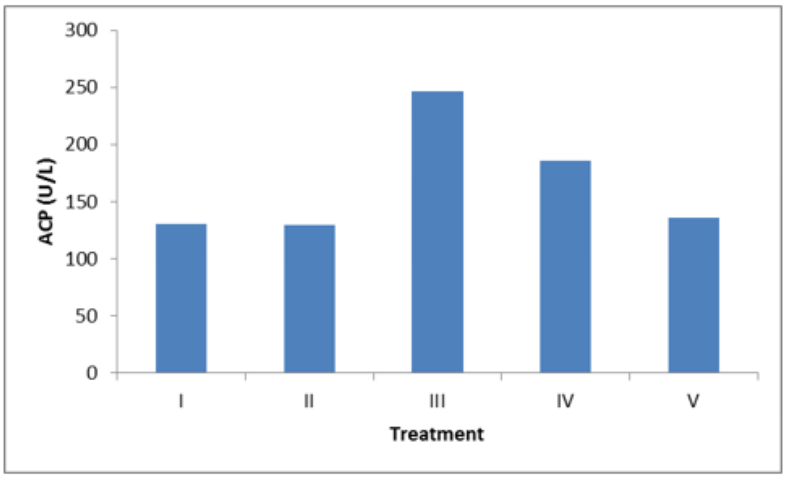

Fig 2.d Level of ACP in various treatment groups

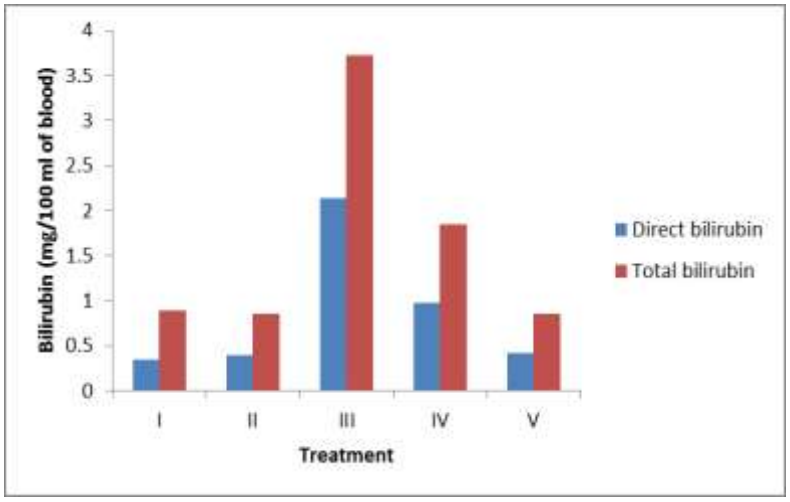

Fig 2e. Level of Bilirubin in various treatment groups

Effects of dietary supplement on liver antioxidant

Cisplatin at a dose of 6 mg/kg body weight was administered to rats for 3 days leads to reduction in the activities of the antioxidant enzymes; Lipid peroxidation (LPO) and endogenous antioxidant systems such as reduced superoxide dismutase (SOD), glutathione (GSH), and catalase in liver homogenate caused the impairment of hepatic functions. As the observations mentioned in Table 3 for Cisplatin reveal, it induces hepatic damage as revealed by significant ($P<0.05$) elevations of liver damage marker enzymes (superoxide dismutase, catalase, and glutathione peroxidase). Cisplatin also caused a significant ($P<0.05$) alteration in plasma. However, there was a significant ($P<0.05$) restoration of the antioxidant status coupled with a significant ($P<0.05$) reduction in the elevated lipid peroxidation, superoxide dismutase, and catalase concentrations after administration of diets containing *Ficus glomerata* fruit (Table 3 and Fig 3a-3d).

Table 3. Effect of diets supplemented with fruits of *Ficus glomerata* on oxidative stress induced by Cisplatin in the liver of experimental animals

GGroup No.	**Treatment**	**Enzymes involved in oxidative stress in liver**			
		LPO (Mole/gm)	**SOD (U/gm)**	**GSH (µMole/gm)**	**Catalase (U/mg)**
I	Normal rats (Basal diets)	65.32±4.17	48.27±5.43	2.89±0.82	6.12±1.24
II	*Ficus glomerata* (4%)+ Normal rats	63.84±2.58	50.19±3.92	2.85±0.42	6.22±1.05
III	Control rats (Cisplatin + Basal diets)	132.47±3.62*	12.53±3.41*	0.31±0.72*	1.08±1.35*
IV	*Ficus glomerata* (2%) + Cisplatin (6 mg/kg)	91.36±2.89[a]	39.92±5.29[a]	1.92±0.68[a]	4.86±1.42[a]
V	*Ficus glomerata* (4%) + Cisplatin (6 mg/kg)	68.25±4.34[a]	55.76±4.36[a]	2.67±0.45[a]	5.98±1.35[a]

Values are expressed as mean SEM, n = 6 in each group. *$P<0.05$ when compared with the normal group and normal *Ficus glomerata* (4%), [a]$P<0.05$ when compared with Cisplatin treated group are considered statistically significant.

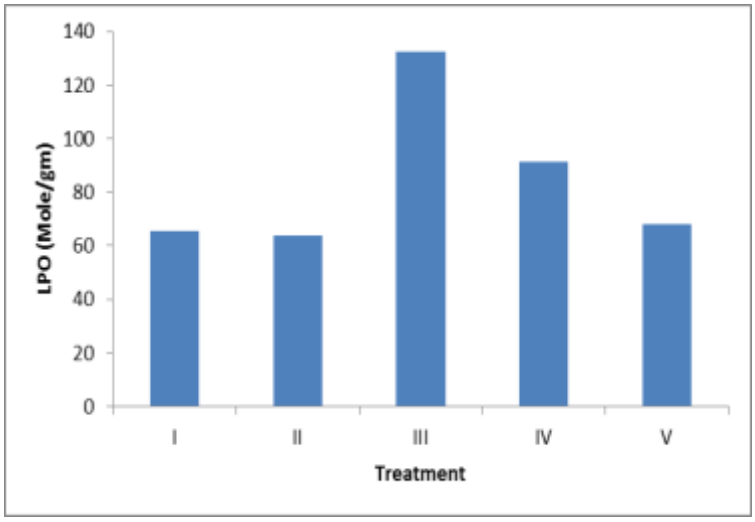

Fig 3a. Level of LPO in various treatment groups

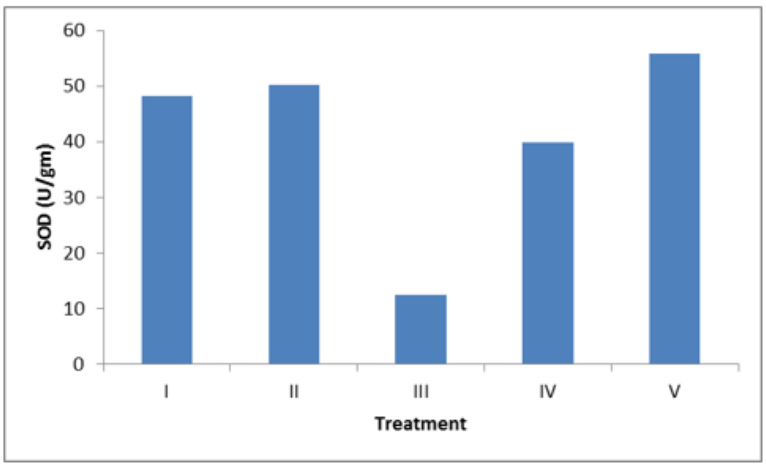

Fig 3b. Level of SOD in various treatment groups

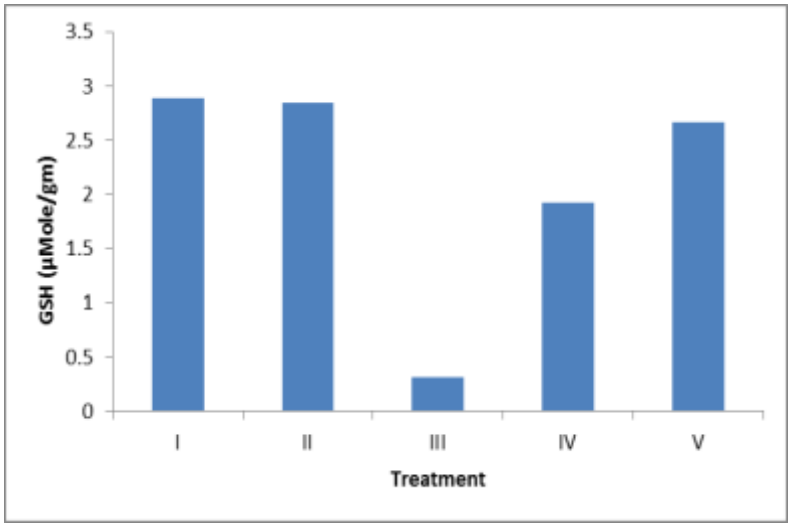

Fig 3c. Level of GSH in various treatment groups

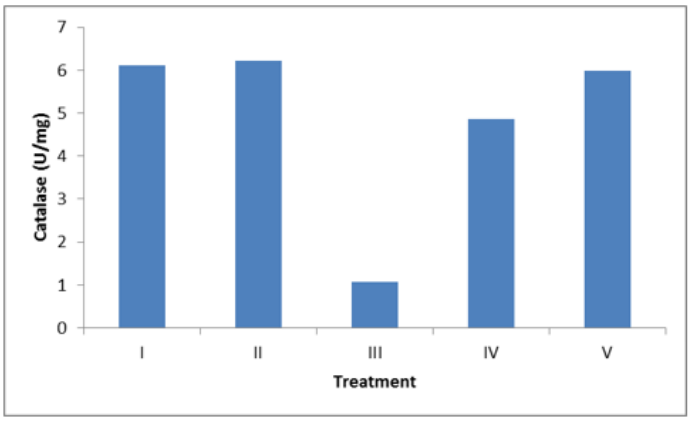

Fig 3d. Level of Catalase in various treatment groups

Histopathological study of liver

Histopathological examination of liver sections of a normal control Group I and 4% *Ficus glomerata* Group II showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and the central vein as shown in Figs. 4a and 4b. Disarrangement of normal hepatocytes with centrilobular necrosis, vacuolization of the cytoplasm, and fatty changes were observed in Cisplatin intoxicated rat livers (Fig 4c). The liver sections of the rats were treated with 2% and 4% of *Ficus glomerata* respectively (Fig. 4d) and (Fig. 4e) showed a sign of protection against Cisplatin intoxication, as evident by the presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein.

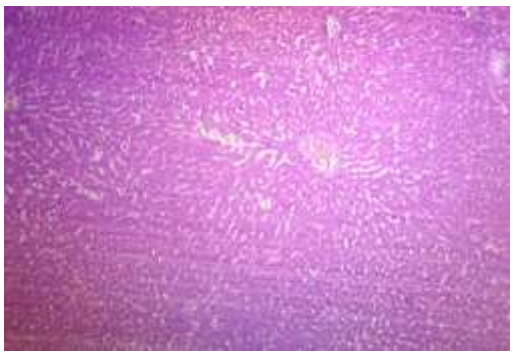

Fig 4a. Microscopically photograph of liver section of normal rats (Group-I)

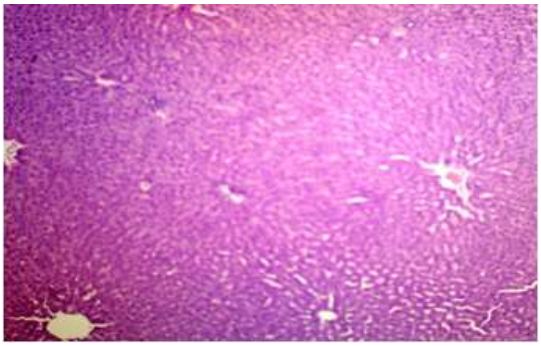

Fig 4b. Microscopically photograph of liver section of normal rats (Group-II)

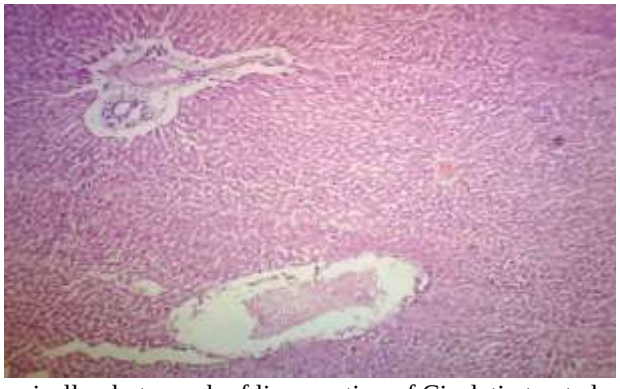

Fig 4c. Microscopically photograph of liver section of Cisplatin treated rats (Group - III)

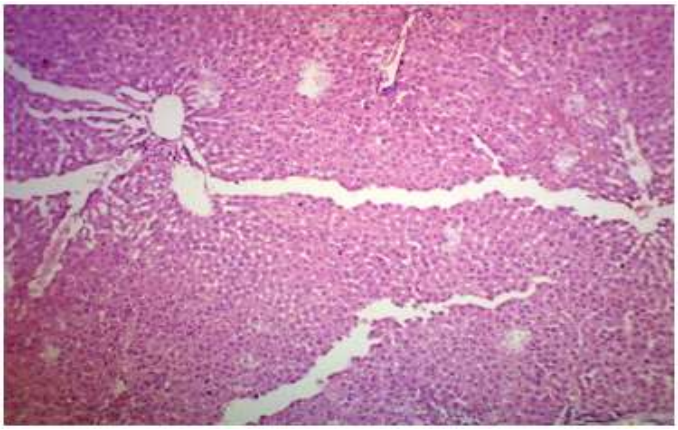

Fig 4d. Microscopically photograph of liver section of Cisplatin + *Ficus glomerata* (2%) treated rats (Group - IV)

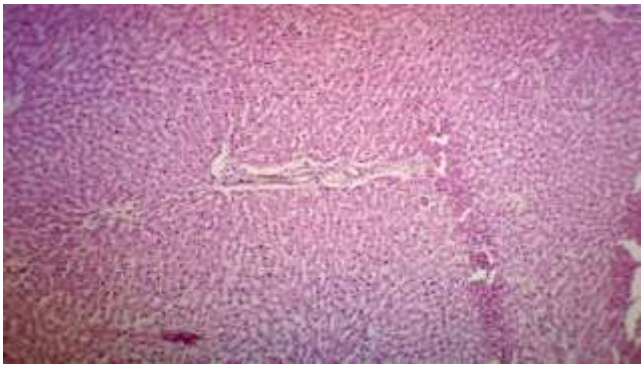

Fig 4e: Microscopically photograph of liver section of Cisplatin + *Ficus glomerata* (4%) treated rats (Group-V)

DISCUSSION

In the present study, cisplatin-induced liver impairment was evidenced by an increase in serum ALT, ALP, and AST and hepatocyte cell degeneration, inflammation, and necrosis. These changes persisted on the 6th day due to a single dose of 6 mg/kg cisplatin. The 2% and 4% of *Ficus glomerata* normalized the serum ALT, ALP, AST, LOD, GSH, SOD, and catalase of the liver. The researchers reported that cisplatin administration induced a significant increase in serum ALT, AST, and ALP and a significant decrease in serum total bilirubin, total protein, and albumin levels. The ability of cisplatin to cause alterations in the activity of these enzymes could be a secondary event following cisplatin-induced liver damage with the consequent leakage from hepatocytes [17, 18, 19]. The present study revealed significantly decreased levels of ALT, ALP, and AST in blood serum due to their antioxidant effects and their ability to act as free radical scavengers, thereby protecting membrane permeability after treatment with 2% and 4% of *Ficus glomerata, which* indicate hepatoprotective and curative effects. Actually, cisplatin contributed to various mechanisms of liver dysfunction consisting of cellular toxicity, vasoconstriction in the renal microvasculature, and proinflammatory effects by producing free radical oxidative stress, which participated in the decline of antioxidant enzyme levels in the liver and led to liver injury. Hence, the possible mechanism of hepatoprotection and curative effect of *Ficus glomerata* supplementation could be due to its good antioxidant potential. It might contribute to free radical scavenging and antagonizing hepatotoxicity that were produced by cisplatin in liver injury [20, 2l, 22].

Cisplatin drug is mostly prescribed for treatment of various benign and malignant tumors like testicular, ovarian and breast. The frequent use of Cisplatin may induce dose dependent liver failure. The oxidative stress and free radicals associated with Cisplatin proposed as the foremost cause for hepatotoxicity. Flavonoids are widely distributed in the *Ficus glomerata* fruit, and exhibit various pharmacological activities including nephroprotective and hepatoprotective activity. The present study was undertaken to explore the modulatory efficacy of dietary inclusion of *Ficus glomerata* fruit against Cisplatin-induced hepatotoxicity in rats. The basal diet supplemented with 2% w/w and 4% w/w powdered fruit pulp of *Ficus glomerata* was prepared separately. Protective activity of the basal diet supplemented with 2% w/w and 4% w/w powdered fruit pulp of *Ficus glomerata* was established on the basis of hepatotoxicity induced by Cisplatin. A liver function test was confirmed on the basis of the levels of SGOT, SGPT, ALP, ACP and Bilirubin. Histology of the liver was also performed. Level of SOD, GSH, LPO and Catalase in liver to establish the modulator potential of basal diet supplemented with powdered fruit pulp from *Ficus glomerata* on oxidative stress. The administration of Cisplatin caused significant changes in the levels of SGOT, SGPT, ALP, ACP, and Bilirubin compared to normal groups, demonstrating the toxic effect on the liver. The pretreatment with basal diet supplemented with powdered fruit pulp of *Ficus glomerata* preserves the changes in biochemical parameters and validates the hepatoprotective effect of diets. Protective Cisplatin led to a significant increase in oxidative stress, suggesting liver damage. The pretreatment with a diet supplemented with powdered fruit pulp of *Ficus glomerata* recovered the levels of SOD, GSH, LPO and catalase and protects the liver from oxidative stress. The possible mechanism hepatoprotective has not been reported yet. It is assumed that the effect of a diet

supplemented with powdered fruit pulp from *Ficus glomerata* on liver protection is related to free radical suppressing activity.

Hence, from the present study, it can be concluded that basal diets supplemented with powdered fruit pulp of *Ficus glomerata* are good sources of hepatoprotective. The improvement in liver function of rats is due to antioxidant status and modulating oxidative stress properties of *Ficus glomerata*. Accordingly, dietary inclusion of *Ficus glomerata* may be a cheap management strategy in the management of acute hepatotoxicity induced by cisplatin.

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CONFLICT OF INTEREST

None

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None

REFERENCES

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