Preliminary Phytochemical Screening, In-Vitro Antioxidant Activity and Hepatoprotective Activity of Root of Ficus religiosa Linn

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ABSTRACT

Since ancient times, *Ficus religiosa*is a used to treat different ailments. The folkloric use of this plant is mentioned as hepatoprotective. So, the present study was designed to assess the *in-vitro* antioxidant activity and the hepatoprotective activity of ethanol and aqueous extract of *Ficus religiosa* Linn against paracetamol induced hepatotoxicity in rats.The different extracts were evaluated for preliminary phytochemical screening and physicochemical analysis. The free radical scavenging activity of ethanol and aqueous extracts of root of *Ficus religiosa* Linn and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. For the evaluation of the hepatoprotective activity paracetamol induced Wistar rats were treated with Silymarin (100 mg/kg, body weight) and aquesous extract of *Ficus religiosa* (150, 300 and 450 mg/kg, p.o.). The estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein was reported. The presence of alkaloids, sugars, proreins, volatile oils, cardiac glycosides etc. were confirmed and the physicochemical parameters such as ash values, extractive values and loss of drying were determined on the powdered root of *Ficus religiosa.* The DPPH free radical scavenging activity of the ethanol and aqueous extracts of roots of *Ficus religiosa* and ascorbic acid showed at different concentrations. The absorbance of various concentrations of the ethanol and aqueous extracts decreases as the concentration increases, as the concentration of the test sample increases the percentage inhibition value also increases. On the other hand, the animals treated with ethanol and aqueous extracts of roots of *Ficus religiosa* showed statistically significant ($p<0.01$) protection against paracetamol induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. The aqueous extract of roots of *Ficus religiosa*low, medium and high dose significantly reversed the levels of SGOT, SGPT, ALP, total bilirubin and total protein when compared to paracetamol alone treated rats. From the above preliminary study, we conclude that the aqueous extract of roots of *Ficus religiosa* are presented to be one of the herbal remedies for liver ailment. Further studies are though recommended to characterize the active principles and to elucidate the mechanism are in progress.

KEYWORDS: *Ficus religiosa*, hepatoprotective, DPPH, anti-oxidant, Silymarin.

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INTRODUCTION

The liver is a vital organ that plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification [1]. Hepatotoxicity (from *hepatic toxicity*) implies chemical-driven liver damage. Drug-induced liver injury is a cause of acute and chronic liver disease. More than 900 drugs have been implicated in causing liver injury responsible for 5% of all hospital admissions and 50% of all acute liver failures[2-4]. Early diagnosis of drug-induced liver reactions is essential to minimizing toxicity though the drugs that produce hepatotoxicity unpredictably, biochemical monitoring is less useful. ALT (Alanin Transferase) values are more specific than AST (Aspartate Transferase) values [5]. ALT values that are within the reference range at baseline and rise 2- to 3-fold should lead to enhanced vigilance in terms of more frequent monitoring. ALT values 4-5 times higher than the reference range should lead to prompt discontinuation of the drug [6, 7].

No specific treatment is indicated for drug-induced liver disease. Treatment is generally supportive and based on symptomatology. About 170 phytoconstituents isolated from 110 plants belonging to 55 families were stated to possess liver protective activity about 600 commercial herbal formulations with claimed hepatic protective activity are being marketed worldwide [8].

Ficus religiosa L.*,* commonly known as Bodhi tree, had derived from the Latin word 'Fig', the fruit of the tree and'*Religiosa*' refers to 'religion' [9]. This tree is sacred in both Hinduism and Buddhism and reported to have numerous therapeutic uses in folk medicine viz.: leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhoea, hematuria, earache and toothache, migraine, eye troubles, gastric problems and scabies. Leaf decoction has been used as an analgesic for toothache, fruits for the treatment of asthma, other respiratory disorders and scabies. Stem bark is used in gonorrhea, bleeding, paralysis, diabetes, diarrhea, bone fracture, antiseptic, astringent and antidote [10-12]. The state-of-the-art review showed that the aqueous bark extract of this plant has antioxidant property as well [13]. So, this present study aimed to assess the in-vitro antioxidant activity and the hepatoprotective activity of ethanol and aqueous extract of *Ficus religiosa* against Paracetamol induced hepatotoxicity in rats by following of OECD Guidelines No.423.

.MATERIALS AND METHODS

Collection and authentication of the plant

The root of *Ficus religiosa* was collected from the local area of Jhalwa, Allahabad, Uttar Pradesh, India in the month of May 2019 and the plant specimen was authenticated by Botanical Survey of India, Central Regional Centre, Allahabad (Ref. No. 104186).

Crude extracts preparation

The root was dried under the shade, powdered with a mechanical grinder and passed through a 40 mesh sieve. Root powder was collected for the determination of the physicochemical parameters. Petroleum ether (PEE), chloroform (CE), ethyl acetate (EAE), ethanol (EE) and aqueous extracts (AE) were prepared by the successive solvent cold extraction method and the solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator (*Buchi Model, Jyoti Lab, Gwaliar, India*).The extracts were kept for the further preliminary screening and pharmacological studies.

Physicochemical and preliminary phytochemical screening

Physicochemical parameters like such as ash values (Total ash values, acid insoluble values and water soluble values), extractive values (Alcohol soluble extractive and water soluble extractive) and loss of drying were determined as per standard methods on the powdered root of *Ficus religiosa* [14].

The yield of all the extracts were weighed in g and calculated as percentage yield (in %w/w). The qualitative preliminary phytochemical tests were performed with all the extracts to confirm the presence of various phytochemicals like alkaloids, steroids, carbohydrates, proteins, amino acids, fats and oils, volatile oils, tannins, phenolic compounds, cardiac glycosides, anthraquinone glycosides and saponin glycosides by the following standard methods. To identify alkaloids in the extracts Dragendorff's, Mayer's, Hager's and Wagner's tests were performed. Salkowski reaction and Liebermann's test were performed to identify steroids. Molish's test (for carbohydrate), Fehling and Benedict's test (for reducing sugar), tannic acid test (for starch), test for gums and mucilage were also performed. Proteins and amino acids were confirmed by Biuret test and ninhydrin test respectively. Standard methods were followed to identify cardiac glycosides, anthraquinone glycosides and saponin glycosides [14,15].

***In-vitro* Antioxidant activity: DPPH radical scavenging activity**

The free radical scavenging activity of ethanol and aqueous extracts of root of *Ficus religiosa* Linn and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. DPPH solution (0.1 mM) in ethanol was prepared and 1 ml of this solution was added to 3 ml of extract solution in water at different concentrations (100-1000 µg/ml). After 35 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenged\ (\%) = A_{cont}\text{-}A_{test} / A_{cont} \times 100$$

Where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts [16,17].

Hepatoprotective activity

Experimental animals

Healthy, adult Albino Wistar rats (180-200gm) of either sex were purchased from the I.V.R.I. Barelly used for study. The Institutional Animal Ethics Committee approved the study (Ref. No. SIP-IAEC/001/04/19).

Acute toxicity study

The acute toxicity study was carried out as per guidelines set by OECD, revised draft guidelines 423, received from CPCSEA, ministry of social justice and empowerment, Govt of India [14]. The animal are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annexure 2 describe the procedure that should be followed for each of the starting doses [18].

Experimental design

The rats were randomized into five groups comprising of six animals in each groups as given below [19].

Group I : Normal control rats given tween 80 for 4 days.

Group II : Rats have been given Tween 80 for 4 days followed by paracetamol (2 gm/Kg, p.o.) on 3rd day.

Group III : Rats have been given Silymarin (100 mg/kg, p.o.) for 4 days followed by paracetamol (2 gm/kg, p.o.) on 3rd day.

Group IV : Test rats have been given AE of *Ficus religiosa*(150 mg/kg, p.o.) followed by paracetamol (2 mg/kg, p.o.) on 3rd day.

Group V : Test rats have been given AE of *Ficus religiosa*(300 mg/kg, p.o.) followed by paracetamol (2 mg/kg, p.o.) on 3rd day.

Group VI : Test rats have been given AE of *Ficus religiosa*(450 mg/kg, p.o.) followed by paracetamol (2 mg/kg, p.o.) on 3rd day.

AE indicates the aqueous extract.

Assessment of Hepatoprotective activity

At the end of 5th day, blood was collected by heart puncture and serum was separated for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein and the liver was isolated from the rats of all the groups and kept in 10% formalin solution and hence send for histopathological investigation [19].

Estimation of SGOT

Add 0.5 ml of SGOT substrate into a test tube marked as test. Keep in water bath at 37ºC for 5 minutes. Add 0.1 ml of serum and mix. Keep the tube in the water bath and incubate for 60 minutes, after 60 minutes add 0.5 ml of 2, 4 dinitrophenyl hydrazine then add 5 ml of 0.4N sodium hydroxide to the test tube and mix well. After 5 minutes read the optical density at 505 nm or using green filter [20].

Estimation of SGPT

Add 0.5 ml of GPT substrate into a test tube labeled as test; keep the test tube in a water bath at 37ºC for 5 minutes. Add 0.1 ml of serum and mix, keeping inside the water bath. Incubate for exactly 30 minutes. Add 0.5 ml of 2, 4 dinitrophenyl hydrazine solution at the end of 3 minutes. After 5 minutes read the optical density at 505 nm or using green filter against water [20].

Estimation of alkaline phosphatase

Mix 1 ml of buffer and 1 ml of substrate in a test tube and place in a water bath at 37ºC for 3 minutes. Add 0.1 ml of serum, mix gently and incubate for 15 minutes. Stop reaction by addition of 1 ml of 0.4N sodium hydroxide [20].

Estimation of total bilirubin

Dilute 1 ml of serum to 20 ml with water in a conical flask and mix. Take two test tubes and label them as test and blank. Add 5 ml of methanol, 1 ml of 1.5% hydrochloric acid and 4 ml of dilute serum into the test tube labeled as blank and mix well. Add 5 ml of methanol, 1 ml of diazoreagent and 4 ml of diluted serum into the test tube labeled as test and mix well. Keep both the test tubes at room tempetature for 30 minutes. After 30 minutes read optical density using green filter or at 540 nm [20].

Estimation of total protein

Peptide bonds of protein in serum react with cupric ions in alkaline solutions to form a blue coloured complex, the absorbance of which was measured at 576 nm. The intensity of blue colour is proportional to the amount of protein present [20].

Statistical analysis

Hepatoprotective activity: the data was represented as ±SEM. Results was analyzed by one way ANOVA followed by Dunnett's multiple comparison tests using graph pad in stat 3.0 software.

RESULTS

Physicochemical and preliminary phytochemical screening

The percentage yield in different solvents is presented in Table 1; highest percentage yield was obtained in the aqueous extract 1.94%w/w as compared to other values.

Table 1: Percentage yields of various extracts of roots of *Ficus religiosa*.

Sr. No.	Solvent for extraction	Extract obtainpercentage (in %w/w)
1.	Petroleum ether extract (PEE)	0.0512
2.	Ethyl acetate extract (EAE)	0.0816
3.	Chloroform extract (CE)	0.1568
4.	Ethanol extract (EE)	0.6876
5.	Aqueous extract (AE)	1.94

The physicochemical parameter such as ash values, extractive values and loss of drying were determined on the powdered root of *Ficus religiosa* and the results were presented in Table 2. Total ash values was found to be 11.5 %w/w. Alcohol and water soluble extractive values were noted as 20.8 and 24.8 %w/w respectively.

Table 2: Physicochemical analysis of roots of *Ficus religiosa*

Sr. No.	Parameter	Percentage(% w/w)
1.	**Ash values**	
	Total ash values	11.5
	Acid insoluble values	4.5
	Water soluble values	9.5
2.	**Extractive values**	
	Alcohol soluble extractive	20.8
	Water soluble extractive	24.8
3.	**Loss of drying**	0.41

Table 3 represented the preliminary studies on the various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, fixed oils, phenolic and tannins.

Table 3: Preliminary phytochemical studies of the extracts of roots of *Ficus religiosa*

Test and reagents	PEE	EAE	CE	EE	AE
Alkaloids	- - + +	+ + - +	+ + + +	+ + - +	+ + + -
Steroids	- -	- -	+ -	- +	- +
Carbohydrates	-	-	-	-	-

Reducing sugars	+ -	+ -	+ -	+ -	+ -
Non-reducing sugars	- +	- -	- +	- -	- -
Gum	-	-	-	+	-
Mucilage	-	-	-	-	+
Proteins	+	+	+	-	-
Amino acid	-	+	-	-	+
Fats and oils	- +	- +	- -	- -	- -
Volatile oils	- + +	- + +	+ - +	+ - +	+ - +
Tannins	-	-	+	+	+
Cardiac glycosides	- - - -	- + - -	- - + -	+ - - +	+ - - +
Anthraquinone glycoside	-	-	+	+	+
Saponin glycosides	- -	- -	- -	+ +	+ +
Flavonoids	-	-	-	+	+

"+"- Presence; "-"- Absence

***In-vitro*Antioxidant activity**

DPPH is frequently used to determine radical scavenging activity of natural compounds and its radical form absorbs at 517 nm due to the antioxidant activity, the absorption decreases may be the formation of its non-radical form such as DPPH–H. The DPPH free radical scavenging activity of the ethanol and aqueous extracts of roots of *Ficus religiosa* and ascorbic acid showed at different concentrations. The absorbance of various concentrations of the ethanol and aqueous extracts of roots was taken and compared with that of the ascorbic acid, that the absorbance decreases as the concentration increases, as the concentration of the test sample increases the percentage inhibition value also increases, the investigated extracts presented that the DPPH free radical scavenging activity and IC_{50} value of ethanol and aqueous extracts of roots were found to be 561.80 and 704.23 µg/ml. The IC_{50} value of ascorbic acid was found to be 537.63 µg/ml. The data was presented graphically in Fig1.

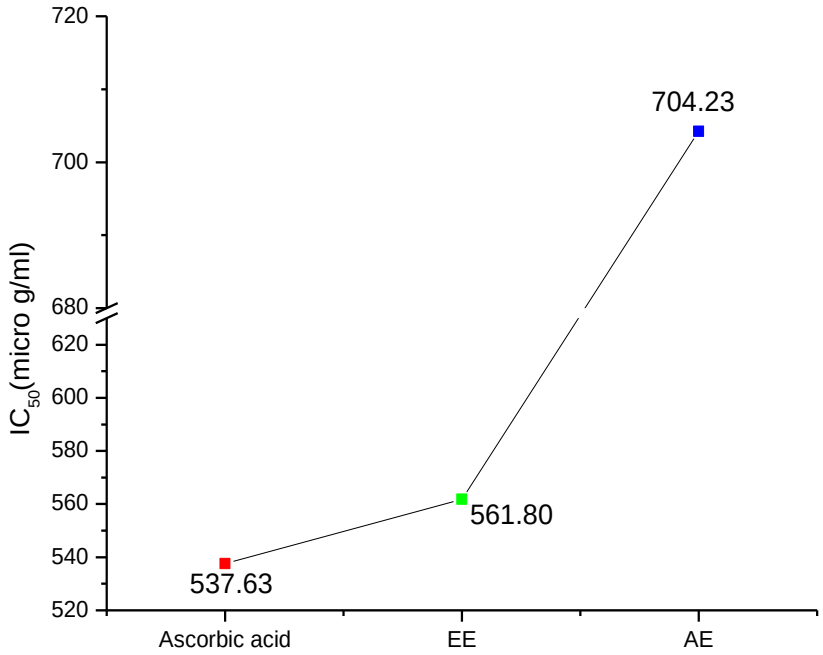

Fig 1: IC_{50} values of ethanol and aqueous extracts of roots of Ficus religiosa compared to standard

Hepatoprotective activity

The hepatic enzymes SGOT, SGPT, ALP, total bilirubin and total protein in serum was significantly increased in paracetamol treated animals when compared to normal. The aqueous extract of roots of *Ficus religiosa* low, medium and high dose significantly reversed the levels of SGOT, SGPT, ALP, total bilirubin and total protein when compared to paracetamol alone treated rats. So the animals treated with ethanol and aqueous extracts of roots of *Ficus religiosa* showed statistically significant ($p<0.01$) protection against paracetamol induced hepatotoxicity in rats, which is comparable to the reference compound Silymarin. Thus Silymarin (100 mg/kg) treated animals showed significant decrease in SGOT, SGPT, ALP, total bilirubin and total protein levels.

Table 4 showed the results of hepatoprotective activity of aqueous extracts of roots of *Ficus religiosa* on paracetamol induced hepatotoxicity in rats. Histopathological studies had shown in Fig 2.

Table 4: Effect of aqueous extract of Root of *Ficus religiosa* Linn on serum marker enzymes (SGOT, SGPT, and ALP), Total bilirubin, total protein and total cholesterol on paracetamol induced hepatotoxicity in rats

Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Total Protein (gm/dl)	Total cholesterol (Mg %)
I	157.28 ± 0.0601**	87.28 ± 0.0601**	168.26 ± 0.0667**	0.654 ±0.0076**	8.86 ± 0.0076**	12.66 ± 0.0058**
II	246.26 ±0.0667	128.26 ± 0.0667	242.21 ±0.0477	6.23 ± 0.0097	4.62 ±0.0058	30.22 ±0.0058
III	162.22 ± 0.0477**	91.21 ± 0.0477**	178.26 ± 0.0667**	0.735 ± 0.0076**	7.848 ± 0.0048**	15.158 ± 0.0065**
IV	171.28 ± 0.0477**	93.28 ± 0.0477**	179.3 ± 0.0577**	0.7433 ± 0.0088**	7.213 ± 0.0049**	17.53 ± 0.0058**
V	174.26 ±0.0667**	98.26 ± 0.0667**	182.28 ± 0.0477**	0.845 ± 0.0076**	6.9566 ± 0.0071**	18.213 ± 0.0061**
VI	164.28 ± 0.0601**	92.28 ± 0.0601**	172.28 ± 0.0601**	0.725 ± 0.0076**	7.82 ± 0.0058**	16.513 ± 0.0049**

*Values are expressed as mean ± SEM for (n=6) rats in each group, when compared to control **p<0.01*

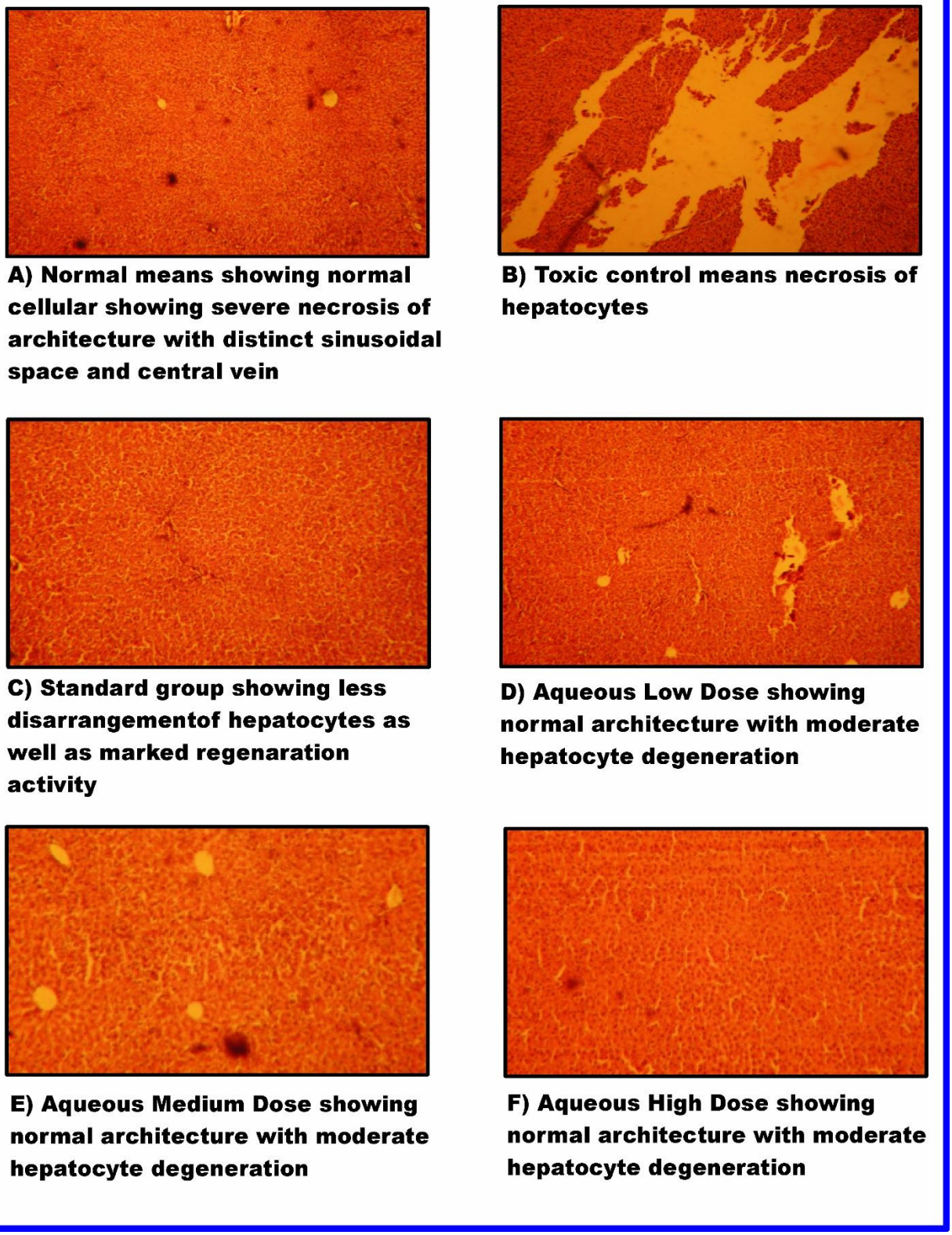

Fig 2:(A-F) Histopathological investigation of aqueous extract of Ficus religiosa Linn.

DISCUSSION

A lot of medicinal plants, traditionally used for thousands of years, are present in group of herbal preparation of the Indian traditional health care system. In India, over 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. Scrutiny of the literature indicates that 160 phytoconstituents from 153 plant families have anti-hepatotoxic activity [21,22].

The phytochemical analysis of roots of *Ficus religiosa* showed high concentrations of saponins, glycosides, moderate concentrations of flavonoids and tannins, low alkaloids, phenols, and steroids. From above data indicated that *F. religiosa* root extracts contained phenolic acids and flavonoids which appeared to be responsible for its antioxidant activity. Recent investigations showed differences between the tests systems for the determination of antioxidant activity are use of at least two methods has been recommended [23]. The hydrogen-radical scavenging action has been known as an important mechanism of antioxidation. DPPH free radical scavenging activity and IC_{50} value of ethanol and aqueous extracts of roots were evaluated as 561.80 and 704.23µg/ml respectively. The DPPH scavenging activity was found to portion subordinate. Consequently extricate has been laid out the as an antioxidant. The diminishing limit fills in as huge sign of cell reinforcement action. The diminishing power expanded with the rising concentration of sample [24].

Liver damage might happen because of natural poisons, drugs, and microbial metabolites. There are two arrangements of catalysts, stage I and stage II proteins which assume a vital part in the digestion and detoxification of different medications and different poisons. Paracetamol is one of the most normally involved drugs as an analgesic and antipyretic; it is a structural derivative of phenacetin, which was removed because of worries for nephrotoxicity [25]. Paracetamol is generally protected contrasted and different NSAIDS; be that as it may, go too far can cause a range of liver wounds from gentle rise in liver proteins to intense liver disappointment and encephalopathy [26].

In the present study, treatment with aqueous extract of *Ficus religiosa* roots suppressed the elevated serum levels of AST, ALT towards the respective normal value this clearly indicates that the plant extract has stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage. Serum ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure [27].

Histopathological studies showed paracetamol to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with silymarinand *Ficus religiosa* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control. The abnormal high level of serum ALT, AST, ALP, protein and bilirubin observed in paracetamol induced liver toxicity. Treatment with aqueous extract of roots of *Ficus religiosa* reduced the enhanced level of serum ALT, AST, ALP, total protein and total bilirubin. On the other hand, the level of liver glycogen of paracetamol treated animals was significantly reduced. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. Protection against paracetamol induced toxicity has been used as a test for potential

hepatoprotective activity by several investigations [28, 29].

The hepatoprotective effect of roots of *Ficus religiosa* aqueous extract against paracetamol hepatotoxicity was reported by which supported the results of this study. Also, the hepatoprotective effect of silymarin against paracetamol hepatotoxicity was confirmed by previous finding of.

CONCLUSION

In present study, the aqueous extracts of roots of *Ficus religiosa* have presented the ability to maintain the normal functional status of the liver. Hepatoprotective activity of the aqueous extracts of roots of *Ficus religiosa* were studied and showed adequate hepatoprotective activity on male albino wistar rats.

Thus is concluded that the extract exhibited significant dose dependent hepatoprotective activity. From the above preliminary study, we conclude that the aqueous extracts of roots of *Ficus religiosa* are presented to be one of the herbal remedies for liver ailment. Further studies are though recommended to characterize the active principles and to elucidate the mechanism are in progress.

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