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ORIGINAL ARTICLE

Iron overload Induced liver toxicity of ethanol extract of *Ficus religiosa* in mice

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ABSTRACT
The Ficus religiosa leaf extract has been used extensively in Ayurveda to treat a wide range of common disorders, including its antimicrobial, anti-parasitic, anti- Parkinson's, anticonvulsant, anti-amnesic, anticholinergic, anti-diabetic, anti-inflammatory, analgesic, cytotoxic, anti-ulcer, wound healing, antioxidant, anti-asthmatic, reproductive, nephroprotective, hepatoprotective and skin protective property. During the present research the hepatotoxicity caused by iron overload was evaluated in the current study using the ethanol leaf extract of F. religiosa. For the finding of iron over load liver toxicity method was given in daily treatment form of F.religiosa extract for the three 7 consecutive days. Light ether anaesthesia was used to sacrifice the animals and they were then dissected. Blood and tissue homogenates were examined for SGOT, ALP, and LPO, reduced glutathione, protein, and catalase in the normal and iron overload groups. The iron over load liver toxicity methods considerably decreased with daily administration of ethanol leaf extract of F. religiosa at dosages of 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight. However, no alteration has been showed in the normal group of the animals at all the doses in research. The ethanol extract also exhibited the significant reduction of hydroxyl radicals in comparison to Desirox 20mg/kg in a dose dependent manner. The consequence have confirmed that the ethanol extract of the Leaf of F.religiosa possess the good antioxidant properties, confirmed by the declination in the level of lipid peroxidation and inclination in the level of reduced glutathione as well as catalase activity in the in-vivo method. This antioxidant property of F.religiosa might be accountable for its significance in traditional medicine system for the cure of various important diseases.
Keyword: *F.religiosa, Hepatoprotective activity, Free radical scavenging activity, Iron Dextran.*

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INTRODUCTION

The liver is an important organ for metabolism and excretion in the human body. Almost every metabolic route leading to growth, disease prevention, nutrition uptake, energy production, and reproduction involves it [1]. Hepatic damage distorts these metabolic processes, sometimes leading to major health issues [2].As the liver is primarily the active location of iron storage in our bodies, hepatotoxicity is the most frequent finding in patients with iron excess [3]. The body's most significant transition metal, iron, is present in functional forms in haemoglobin, cytochromes,myoglobin, enzymes with iron sulphate complexes, and other iron-dependent enzymes [4].Although cells always maintain an ideal level of iron to maintain a balance between essentiality and toxicity, there are situations when this balance is upset, leading to iron overload, which is related to oxidative stress-induced diseases like anaemia, liver cirrhosis, heart failure, fibrosis, diabetes, depression, arthritis, impotence, cancer and infertility[5]. Chelation therapy, which removes iron from the body, is a successful life-saving procedure in all disorders carried by iron overload. Deferoxamine, 1,2-dimethyl-3-hydroxypyrid-4-one (deferiprone), and deferasirox are the three iron-chelating medications that are now accessible and utilised clinically. However, these substances exhibit a number of drawbacks and adverse effects [6, 7], which point to the need for a more potent and secure medication [8, 9] that could improve the therapeutic advantages for patients.

The most significant phytoconstituents for treating the hepatic disorders are phenolics and flavonoids. The majority of them have been discovered to be powerful antioxidants [10, 11], and iron chelation is a core part of their antioxidant action [12]. Therefore, a major area of research in hepatoprotection is finding crude medicines with plant origin that have potent antioxidant properties.

Originally *F.religiosa* (syn.) (family *Moraceae*) is native of the Asia-Tropical (India, Bangladesh, Nepal, Pakistan, Myanmar, China, Vietnam Iraq and Thailand) and it is grown in extensive tropical areas[13].It is used in several systems of traditional medicine in India to cure various human diseases and disorders. It is also known as papal or pipul. The leaf juice has traditionally been used to cure a variety of conditions, including dermatitis, ear infections, migraine headaches, ear and tooth pain, and sexual dysfunction. For toothaches, the leaf decoction was employed.

The fruit was used to treat scabies, as well as various respiratory conditions and asthma. The stem bark was employed as an antiseptic, astringent, antidote, in the treatment of gonorrhoea, haemorrhage, paralysis, diabetes, diarrhoea, and bone fracture[14]. The plant included tannins, phenols, saponins, sugars, alkaloids, methionine, terpenoids, flavonoids, glycosides, and steroids, according to phytochemical study [15–17]. This plant has a long history of usage in traditional medicine and is claimed to have antibacterial and antimicrobial properties [18], Anti-diarrheal activity [19], Anti-parasitic activity [20], Anti-Parkinson's [21], Anticonvulsant [22], Anti-amnesic effect [23], Acetylcholinestrase inhibitory effect [24], Hepatoprotective [25] , Nephroprotective[26] Dermato protective effects[27], Antidiabetic [28], Anti-inflammatory and analgesic effects [29, 30], Cytotoxic effect [31], Anti-ulcer [32], Wound healing effect [33], Antioxidant [34], Anti-asthmatic[35], Reproductive effect [36], Therefore, the purpose of this research was to determine when giving *F. religiosa* to mice would normalise the liver damage occurred by an excess of iron.

MATERIAL AND METHODS

Collection of plant and extract preparation

The *Ficus-religiosa* leaves were identified and collected from the Meerut in the months of June - July. The Department of Botany at IFTM University Moradabad will validate the specimens. Dr. Ashok Kumar identified and verified on March 12, 2021.

The leaves were chopped up into tiny pieces and allowed to dry at room temperature for 15 days before being ground into a fine powder and utilised for extraction. Using a Soxhlet apparatus and ethanol (99.9%) for 24 hours, the powdered substance was extracted (55-56^{o}C). Both before and after the extraction, Marc was entirely dry. After the extraction process was complete, the solvent was removed by using the distillation, Afterthat a dark green residue obtained, that was then placed in a desiccator. In respect of dried powder, the percentage yield was obtained 16 percent w/w. Alkaloids, citric acid, maleic acid, and vitamin C were all found in the ethanol leaf extract after a qualitative examination. The dried extract so obtained was evaluated for antioxidant activity using the different method [37].

Chemicals

All of the chemicals utilised in the study, such as petroleum ether, chloroform and ethanol, were of analytical quality.

Animals

In this study, albino mice weighing 20-30g were used. They were maintained in a clean propylene casing under standard laboratory settings (25$\pm$ 2°C, a continuous 12-hour dark/light cycle). Normal laboratory pellet diet and water *ad libitum* given to the animals. All experiments were approved by the institutional animal ethics committee (IAEC) and the care of the animals was provided in according to the guidelines established by the committee for the purpose of control and supervision of experiments on animals (CPCSEA) of the Ministry of Environment and Forest, Government of India. The animals spent one month to getting acclimatized for lab condition before performing the experiment.

Experimental design

A total of 36 mice were divided into 6 groups, each with 6 mice. **One group** served as the normal and received normal saline. The **remaining 5 groups received 5 doses** (one dose every two days) of iron dextran saline, 100 mg/kg b.w. each (i.p). One iron dextran group received normal saline (**Control group**) and other four groups were orally administered with 50 mg/kg, 100 mg/kg, 200 mg/kg plant extract and 20 mg/kg Desirox respectively, for three consecutive 7 day periods, started from the day after the first iron dextran injection.

Histopathology

The experiment was completed on the twenty-first day and the mice were fasted overnight. Blood samples were taken and the plasma was separated to estimate the levels of SGOT, ALP, and albumin. The animals were sacrificed under a light ether anesthesia after that dissected. The liver was taken out,

weighed and preserved in saline water. Then it was homogenized in 8 ml of ice-cold 0.9% saline for 5 minutes using glass homogenizer.The homogenate was then centrifuged for 10 minutes at 4^0C using a 4000rpm machine. Protein (39), LPO (40), Catalase (41) and reduced glutathione were all estimated by using this homogenate.

Statistical Analysis

The results of the hepatoprotective activity were shown as mean ± SEM. Graphpad prism software version 5.0 was used to generate the graph and do the static analysis. Results documentation was displayed as mean ± SEM. For the statistical analysis of the data group means, one-way analysis of variance (ANOVA) was used, followed by the Dunnett's test, where p 0.05 and p 0.01 were considered significant.

RESULTS

For the iron over load group in the liver toxicity model, the statistical significance of the various drug-treated groups was analysed using the Dunett,s test.

Based on the figure, the iron overload group in the liver toxicity model had much higher levels of lipid peroxidation than the normal group.

And there is a remarkable decrease in level of lipid peroxidation in a dose dependant manner with the ethanol extract of leaf of *F.religiosa*, which was significant at $p<0.05$.

At the dose 50mg/kg of ethanol extract of leaf of *F.religiosa* there was very small alterations was occur in the lipid peroxidation level. The outcome were also comparable to Desirox (20 mg/kg) which was taken as standard. The level of Glutathione levels in the iron over load group in liver toxicity model was significantly decreased in mice ($P<0.001$) compared with normal group. Treatment with ethanol extract of leaf of *F.religiosa* at the dose of 50mg/kg, 100mg/kg & 200mg/kg had remarkable effect on increased glutathione levels, the P values ($P < 0.001$) were considered highly significant dose dependent manner.

The level of Total protein levels in iron over load group in liver toxicity model was considerably reduced in mice ($P<0.001$) likened with normal group. Treatment with ethanol extract of leaf of *F.religiosa* at a dose of 50mg/kg, 100mg/kg & 200mg/kg had notable effect on increased Total protein levels, the P values ($P < 0.001$) were considered highly significant dose dependent manner.

The CAT levels in iron over load group in liver toxicity model in mice was remarkably decline in the mice ($P<0.001$) compared with normal group. Treatment with ethanol extract of leaf of *F.religiosa* at a dose of 50mg/kg, 100mg/kg & 200mg/kg had notable effect on increased CAT levels, the P values ($P < 0.001$) were considered highly significant dose dependent manner.

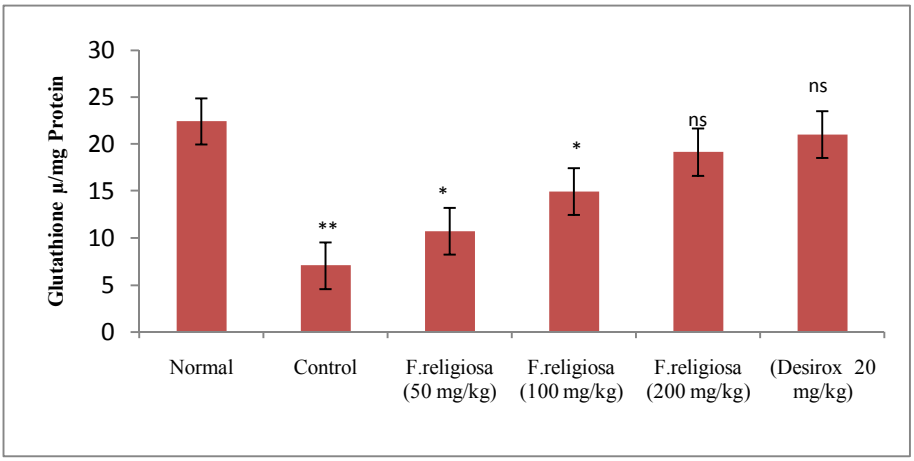

Fig.No-1Effect of various treatment groups on glutathione in iron over load liver toxicity

Results are expressed as mean ± SEM n= 6, ns represent the non-significant changes * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes compared with normal mice by one way Anova followed by Dunett test

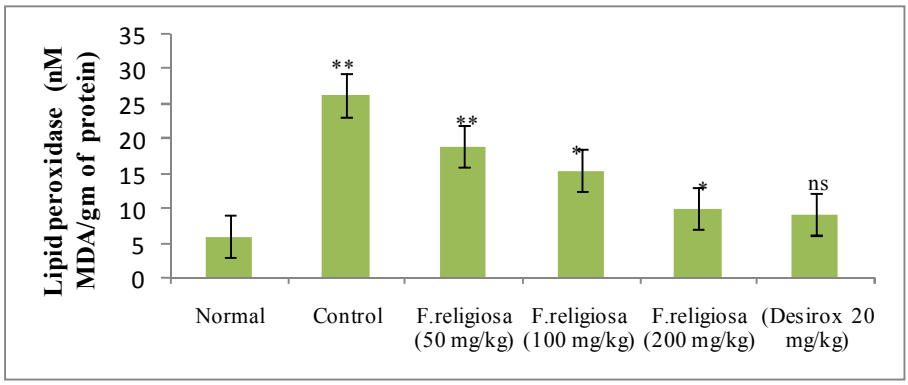

Fig.No-2Effect of various treatment groups on Lipid peroxidase in iron over load liver toxicity

Results are expressed as mean ± SEM n= 6, ns represent the non-significant changes * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes compared with normal mice by one way ANOVA followed by Dunett test

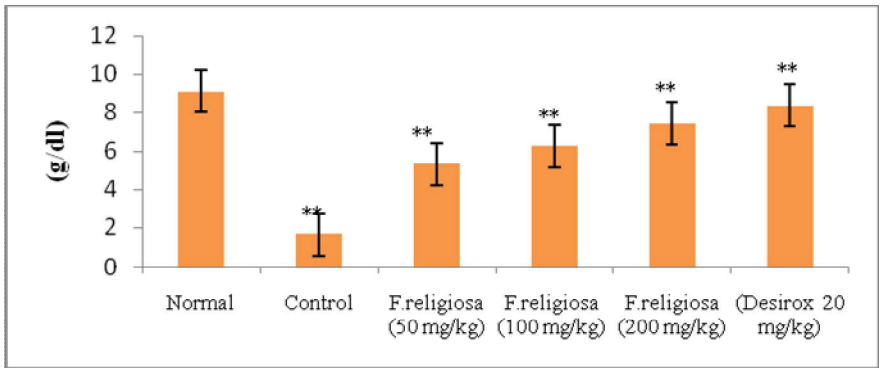

Fig.No-3: Effect Of Various Treatment Groups On Total Protein In Iron Over Load Liver Toxicity

Results are expressed as mean ± SEM n= 6, ns represent the non-significant changes * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes compared with normal mice by one way ANOVA followed by Dunett test

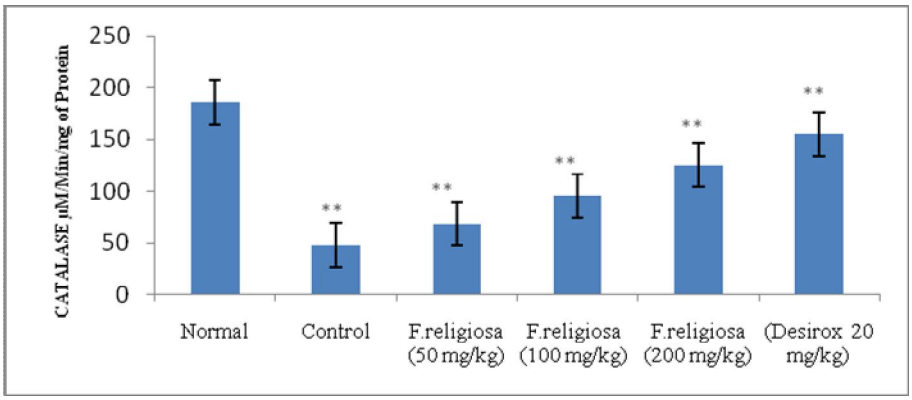

Fig.No-4: Effect Of Various Treatment Groups On Catalasein Iron Over Load Liver Toxicity
Results are expressed as mean ± SEM n= 6, ns represent the non-significantchanges * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes compared with normal mice by one way ANOVA followed by Dunett test

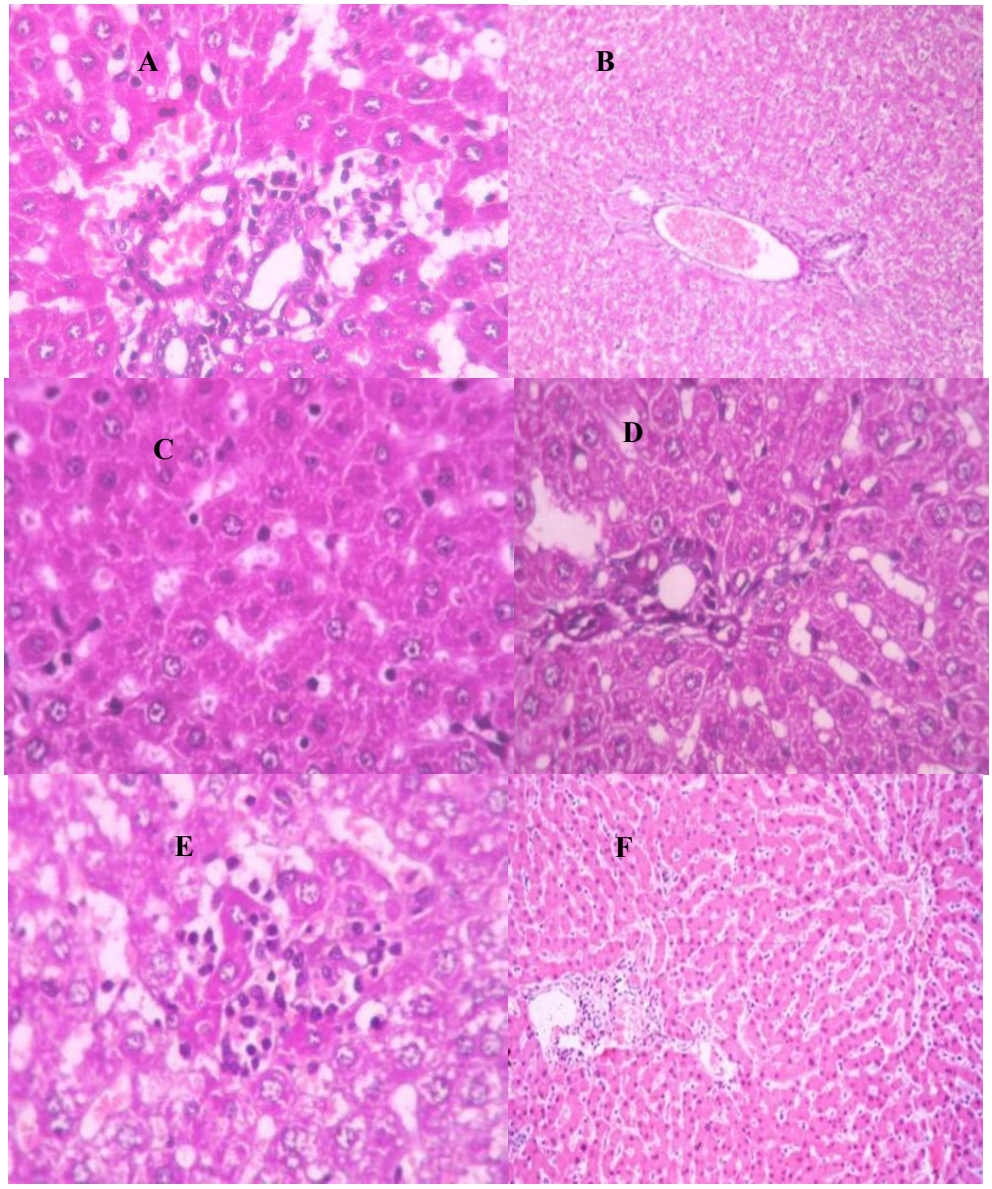

Fig.No- 5 Histopathological changes in mice liver apexes. A: Normal group, B: Iron Dextran (100mg/kg)(Control), C, D&E(Test1, 2 &3): *F.religiosa*(50 mg/kg, 100mg/kg and 200 mg/kg) respectively and F: Desirox (20 mg/kg) (standard).

Histopathology Reports

The hepatic tissues of treated mice from the different groups were examined for histopathological evaluation and their effects were indicated in following listed histopatological figures. A sample of this group's liver tissue demonstrates Hepatocytes that have been revealed are found in cards, trabeculae, and a sinusoidal pattern. The morphology of hepatocytes is normal, with a circular nucleus containing fine chromatin and a moderate amount of eosinophilic cytoplasm. The position and configuration of the portal, central, and hepatic vein triads are normal in the normal control group (A). In the section B, Hepatocyte degeneration was seen with the patchy as well as perivascular inflammation component made up of lymphomononuclear cell aggregates In the toxicant ferrous sulphate group, there is a slight central vein dilation and localised fibrosis has also seen (B).

The liver section exhibits the degeneration in hepatocytes that still exist but with little inflammation. In *F. religiosa* 50mg/kg (C), the bile duct, central vein and portal triad are all quit normal and the liver section of this group exhibits the ablation of fibrous septae and hepatocytes that appear to be normal. The mononuclear inflammatory infiltration of *F. religiosa* 100mg/kg is quite low (D). Hepatocytes that are less deteriorated and have little inflammation are still present in Section E. Central vein, portal triad, Bile duct are finding normal in *F.religiosa* 200mg/kg (E) as well as the liver tissue also show the hepatocytes are

more normal in pattern and arrangement of the cell. There is no inflammatory component was found in the liver section of this group. No thrombosis has been found. Central vein, Portal triad and the bile duct are remarkably normal in Desirox (F) group (standard group).

Table1:Effect of different treatment group on SGOT, ALP and Albumin in Iron over load induced liver toxicity model

Groups	SGOT(U/L)	ALP(IU/L)	Albumin(g/dL)
Normal group	8.34± 3.59	176.36 ± 2.53	4.69 ± 0.96
Control group	88.57±6.96**	559.48 ± 8.62**	1.67 ± 0.93**
F. religiosa (50 mg/kg)	35.14 ± 5.48**	365.22 ± 8.36*	3.12 ± 0.07**
F. religiosa (100 mg/kg)	29.39 ± 6.07**	349 .04 ± 0.50**	3.84 ± 0.24**
F. religiosa (200 mg/kg)	30.10 ± 17.14*	274.42 ± 0.30ns	4.42 ± 0.30**
Standard group (Desirox 20mg/kg)	25.36± 6.52ns	216.63 ± 1.75ns	4.52 ± 0.17**

Results are expressed as mean ± SEM n= 6, ns represent the non-significant changes * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes were highly significant changes compared with normal mice by one way Anova followed by Dunett test

Table2:Effect of different treatment group on LPO, GSH, Protein and Catalase in Iron over load induced liver toxicity model

Groups	LPO(nM MDA/g of Protein)	GSH (µ/mg of protein)	Protein (mg/dl)	Catalase (µM/Min/mg of Protein)
Normal group	6.25±1.60	22.45 ± 6.81	9.09 ± 1.66	185.37±21.72
Control group	26.31±4.56**	7.05 ± 0.98**	1.67± 1.16**	48.37± 18.74**
F.religiosa (50 mg/kg)	19.07 ±2.67**	10.73 ± 2.95**	5.32± 0.97**	68.79±15.28**
F.religiosa(100 mg/kg)	15.53±4.55*	14.94 ± 3.62**	6.24± 0.47**	96.23±6.93**
F.religiosa (200 mg/kg)	10.13 ±1.48ns	19.15 ± 6.16*	7.42± 1.11**	125.57±7.23**
Standardgroup (Desirox 20mg/kg)	9.17 ±4.40ns	21.02 ± 5.40ns	8.35± 1.23**	155.24±11.46**

Results are expressed as mean ± SEM n= 6, ns represent the non-significant changes * p ≤0.05 represent less significant changes, ** p ≤0.01 represent significant changes were highly significant changes compared with normal mice by one way Anova followed by Dunett test

DISCUSSION

One of the primary causes of liver disease is metal-induced liver toxicity. The principal cause of iron-induced liver damage is the Fenton reaction, which produces a hydroxyl radical from hydrogen peroxide.This hydroxyl radical raises the level of lipid peroxidation while lowering glutathione and catalase levels. The current study's results demonstrated that when the liver is overloaded with iron, lipid peroxidation significantly increases and catalase, protein, and glutathione levels in the liver are reduced (42). According to numbers of studies, the chemical induced liver damage may found because of thedisruption of pro- oxidant and antioxidant balance that are occurred in the cells. During this investigation, the estimation of oxidant- antioxidant parameters was performed in blood, because blood is a finest indicator of the alteration in metabolite as well as energy metabolism associated enzyme activity(43). Malondialdehyde, an final product of the lipid peroxidation reaction, is highly utilized as the marker of the lipid peroxidation. Another important enzyme is the Glutathione peroxidase which plays the vital role in the removal of the hydrogen peroxide as well as lipid hydroperoxide inside the liver cells. Nowa days, there is a consent that earlierlethal effects of iron on liver cells are the results of the increased lipid peroxidation and reduction in the glutathoione (GPx) level. (44).

During thecurrent research the antioxidant potency of ethanolic extract of *F.religiosa*leaf was investigated. From the research it was also estimated to declination in lipid peroxidation and significant inclination in the glutathioneprotein and catalase with the ethanolic extract of the *F.religiosa*leaf in dose dependant way. As per the review Literature theethanolic extract of *F.religiosa* leaf contain the higher number of active phyto-constituents such as phenols,tannins, saponins, alkaloids, sugar, terpenoids, glycoside, flavonoids and steroids. Meanwhile all these active constituents are also available in the ethanolic extract of this plant's leaf and possibly thesephytoconstituents are may be associated with the

antioxidant activity of the plant species. Consequently, it was determinedthat the ethanolic extract of *F.religiosa* leaf possessesthe potent antioxidant properties as well as thegood free radical scavenging properties also.

CONCLUSION

The result envisaged from the research have showed that the ethanolic extract of the*F.religiosa*,s leaf having the potent antioxidant activity, validated via the reduction in lipid peroxidation level and inclination in level of the reduced glutathione as well as catalase level during the *in vivo* method. The *in vitro* method's free radical scavenging activities have supported the *in vivo*antioxidant activities' research .These antioxidant properties of *F.religiosa*might be accountable for its significance in traditional medicine system for the cure of the various maladies. The current study exposedthe variousnovel areas of the research work. This research work can be continuous in the future to investigate and verify the liver protective activity in various experimentalmodels as well as also to isolate, identify, characterize as well as standardise the active constituents (s) that are accountable for this liver protective activity.

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