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RESEARCH PAPER

HEPATOPROTECTIVE ACTIVITY OF THE METHANOLIC EXTRACT OF *FAGONIA INDICA* BURM. IN D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN ALBINO RATS

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The aim of the study was to investigate the hepatoprotective activity of the methanolic extract of *Fagonia indica* Burm. on D-galactosamine (D-GalN)-induced hepatotoxicity in albino rats. Animals in Group 1 served as vehicle control, Group 2 served as the hepatotoxin (D-GalN, 400 mg/kg, *i.p.*) treated group, Group 3 served as the Standard (Silymarin 50 mg/kg, *p.o.*) treated group. Groups 4 and 5 served as methanolic extract of *Fagonia indica* (MEFI) in different doses (200 mg/kg and 400 mg/kg *b.w., p.o.*). The degree of protection was determined by measuring levels of biochemical markers. The histopathological studies also showed the hepatic protection of the test extracts. The levels of the biochemical parameters such as SGPT, SGOT, ALP, total bilirubin, direct bilirubin and cholesterol were significantly increased in D-GalN treated rats when compared with the normal group ($P<0.05$), but the MEFI (400 mg/kg, *b.w.*) treated rats showed a maximum reduction of SGOT (85.00±1.23), SGPT (145.83±1.57), ALP (126.16±1.49), total bilirubin (1.73±0.12), direct bilirubin (0.75±0.009) and cholesterol (181.33±1.89) in a significant manner. Histopathological studies also revealed the hepatoprotection property of MEFI in a dose-dependent manner. These results suggested that that MEFI in different doses showed significant hepatoprotective activity against D-GalN-induced hepatotoxicity and this might be due to the presence of flavonoids and tannins. Further research is sought to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

Key words: *Fagonia indica*, Hepatoprotective, D-galactosamine (D-GalN), Silymarin, Histopathology.

INTRODUCTION

Herbal drugs have been used as remedies for a wide array of diseases across the world since ancient times. In recent years, increasing interest has been focused on phytomedicines as safer and more agreeable to the human body. Medicinal plants come into the preparation of various drugs singly or in combination or are even used as the principal source of raw material for other medicines [1-5]. The liver is the most important organ in terms of biochemical activity in the human body. The liver has a great capacity to detoxify and synthesize useful substances, and therefore, damage to the liver inflicted by hepatotoxic agents has grave consequences [6].

Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality. The principal causative factors for liver disease in developed countries

are excessive alcohol consumption and viral-induced chronic liver disease while in developing countries, the most frequent causes are environmental toxins, parasitic disease, hepatitis B and C viruses and hepatotoxic drugs (certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride, thioacetamide etc [7-10].

In liver disorders, the ability of the natural antioxidant system is impaired. Free radicals are generated in cells by environmental factors such as ultraviolet radiation, pollutants, and X-rays, as well as by normal metabolism. Free radicals induce an oxidative state that can lead to cellular membrane injury with consequent alteration in the metabolic process. Reactive oxygen species (ROS) play an important role in the pathogenesis of various degenerative human diseases and have been implicated in atherosclerosis, liver disorders, lung and kidney damage, ageing and diabetes mellitus [11].

Fagonia indica Burm. is one such green plant that is abundantly grown and used as an important medicinal plant (**Figure 1**). However, much of its medicinal importance is not assessed. By extensive literature survey, it is found that the leaves of the plant contain secondary metabolites such as quercetin, kaempferol, isorhamnetin-α-3-O rhamnoside, quercetin 3-O-β-D-glucopyranosyl-(1''-6''')-β-D glucopyranoside and quercetin 3-O-β-D-galactopyranosyl-(6''-1''')-α-L-2'''-acetyl rhamnose-(3'''-1'''')-β-D-glucopyranoside. It also contains triterpenoids, saponins, alkaloids, coumarins, flavonoids and tannins. This plant contains flavonoids hence, it was planned to study its antioxidant and hepatoprotective properties. Free radicals cause organ toxicities which are well reported. Therefore, there is a possibility that the antioxidants may have a protective role. Keeping this in view, it was thought that the plant *Fagonia indica* Burm. which is abundantly grown and used as a medicinal plant may have a protective role in organ toxicities induced by D-galactosamine (D-GalN) induced-hepatotoxicity [12].

Fig. 1. The medicinal plant - *Fagonia indica* Burm. [12]

MATERIALS AND METHODS

Plant material

The plant *Fagonia indica* Burm. was collected from Mandvi, Kutch, Gujarat. The plant was identified and authenticated by Dr P. S. Nagar, Department of Botany, The Maharaja Sayajirao University, Vadodara, Gujarat, India.

Extraction preparation

The plant was shade-dried separately at room temperature and pulverized. The plant product was powdered and subjected to Soxhlet extraction using 70% methanol, resulting extracts were evaporated in a vacuum to dryness and the percentage yields of corresponding extracts were calculated. The extracts were used for *in vivo* hepatoprotective activity studies, after subjecting it to preliminary qualitative phytochemical studies.

Chemicals

The D-galactosamine was collected from Sigma Aldrich, and Silymarin was collected from Microlab Pvt. Ltd, Bangalore, Karnataka, India.

Animals

Albino rats (Wistar) weighing 150-200 g of either sex, were used in this study. They were procured from Shree Dhanvantary Pharmacy College, Kim, Suart, Gujarat, India. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at (25±3˚C) and relative humidity (50±20%) under 1 and water *ad libitum* was provided. The husk in the cages was renewed daily to ensure hygiene and maximum comfort for animals. All experimental animals are conducted according to the guidelines of the CPCSEA.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on 70% MEFI for qualitative identification of the type of phytoconstituents

present. The tests for common phytochemicals were carried out by following standard methods described in the literature [13-14].

Determination of acute toxicity (LD_{50})

The acute toxicity for 70% MEFI was determined on an albino female rat, maintained under standard conditions. The animals were fasted overnight before the experiment. The fixed-dose method of OECD guideline No. 420 given by CPCSEA was adopted for toxicity studies [15].

Evaluation of hepatoprotective activity in D-galactosamine-induced hepatotoxicity

In the dose-response experiment, albino rats were randomly assigned into 5 groups of 6 individuals each. Group I: Animals (-ve control) were administered 1 ml normal saline *p.o.*, for 10 days. Group II: Animals (+ve control) were administered 1 ml normal saline *p.o.*, for 10 days. Group III: Animals were administered with silymarin 50 mg/kg *p.o.*, for 10 days. Group IV: Animals were administered with 70% methanolic extract 200 mg/kg *p.o.*, for 10 days. Group V: Animals were administered with 70% methanolic extract 400 mg/kg *p.o.*, for 10 days. Groups II, III, IV and V received D-GalN 400 mg/kg *i.p.*, on the 9th and 10th day, after 30 min of vehicle, 50 mg/kg silymarin, 200 mg/kg 70% MEFI and 400 mg/kg 70% MEFI administration. Animals were sacrificed on the 11th day under mild ether anaesthesia. Blood samples were collected by retro-orbital plexus route for evaluating the serum biochemical parameters like SGOT, SGPT, ALP, total bilirubin, direct bilirubin, and cholesterol triglyceride. The liver samples were dissected out, blotted off blood, washed with saline and also stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically [16].

Histopathology

Small pieces of liver tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under a microscope for the analysis of the altered architecture of the liver tissue due to D-galactosamine challenge and improved liver architecture due to pretreatment with test extracts and standard drug [17].

Statistical analysis

Results were expressed as mean SEM with (n=6). Statistical analyses were performed with one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using Graph Pad Instat Software. A p-value less than 0.05 was considered to be statistically significant *P<0.05, **<0.01 and ***<0.001, when compared with the control and toxicant group as applicable.

RESULTS AND DISCUSSION

Preparation of extracts and properties

The methanolic extract was obtained by extracting the whole plant with 70% methanol by Soxhlet extraction. The color of the methanolic extract was found to be a dark greenish colour and the percentage yield was calculated as 6% *w/w*.

Preliminary phytochemical screening

The qualitative chemical investigation of MEFI was carried out to check the presence of various phytoconstituents as per standard methods. The results are summarized in **Table 1**. It is observed from the phytochemical study that flavonoids, tannins, alkaloids and saponins are present in the extracts.

Table 1. Preliminary phytochemical investigation of MEFI

Types of phytochemical constituents	70% Methanolic extracts
Alkaloids	+
Flavonoids	+++
Tannins	+++
Saponins	+++

+ indicates presence, +++ indicates better response

Acute toxicity (LD_{50}) studies

An attempt was made to identify the LD_{50} of MEFI. Acute toxicity studies were carried out according to OECD guidelines 420 (Up and Down method). Hence, no mortality was observed at 2000 mg/kg in rats. It was thought that 2000 mg/kg was the cut-off dose. Therefore, 1/10th and 1/5th (200 mg/kg, 400 mg/kg) dose was taken as effective doses for all further *in vivo* studies.

Effect of MEFI on liver biochemical markers in D-GalN induced hepatotoxicity

A significant increase (p<0.0001) in serum SGPT, SGOT, ALP, total bilirubin, direct bilirubin, cholesterol, and triglyceride levels was observed in animals treated with D-GalN (400 mg/kg *i.p.*) as compared to normal. Pretreatment with MEFI (200 mg/kg and 400 mg/kg *p.o.*) for 10 days, decreases the above parameters significantly (p<0.0001) as compared to D-GalN treated group. Silymarin pretreatment produced a significant decrease (p<0.0001) in the above parameter when compared to the D-GalN treated group (**Table 2** and **Figures 2-8**).

Table 2. Effect of MEFI on enzyme SGOT, SGPT, ALP, total direct bilirubin, cholesterol, and triglyceride levels in blood serum of D-GalN induced hepatotoxicity

Groups	Treatment	SGPT IU/L	SGOT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl	Cholesterol mg/dl	Triglyceride mg/dl
Group I	Normal	40.83±1.22	43.00±1.55	94.00±4.16	0.86±0.21	0.23±0.01	135.00±3.27	162.00±1.62
Group II	D-GalN	194.00±8.81	106.66±4.17	174.83±14.27	2.38±0.23	1.56±0.19	224.5±1.72	204.66±1.78
Group III	Silymarin	137.66± 6.77***	74.33± 3.16***	97.33± 5.29***	1.63± 133**	0.43± 0.18***	165.16± 1.55***	170.66± 2.17***
Group IV	MEFI 200 mg/kg	164.16± 0.90**	96.5± 0.56*	146.83± 1.24*	1.94± 0.01^{ns}	0.91± 1.007**	200.66± 1.80***	190.16± 1.90***
Group V	MEFI 400 mg/kg	145.83± 1.57***	85.00± 1.23***	126.16± 1.49***	1.73± 0.12*	0.75± 0.009***	181.33± 1.89***	179.16± 2.30***

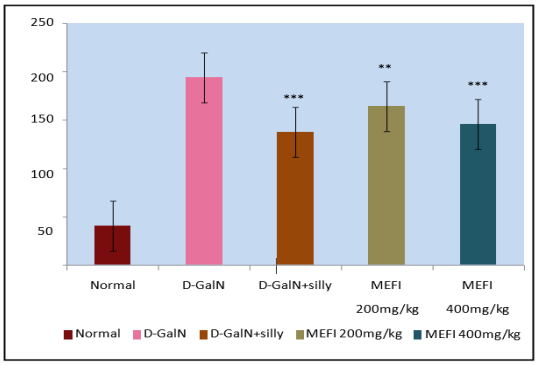
Each value represents Mean ± SEM. n=6, Values in the parentheses indicate 'p'value.*P<0.01; **p<0.001;***p<0.0001; ns= not significant compared to D-GalN group. One-way ANOVA Followed by Dunnett's multiple comparison tests.

D-GalN-induced hepatotoxicity resembles both morphologically and functionally viral hepatitis in humans. D-GalN administration in rats leads to disruption of the membrane permeability of the plasma membrane [18]. These cause leakage of enzymes from cells leading to elevation in the level of the serum enzyme. Elevated serum enzymes are an indication of cellular leakage and loss of functional integrity of the cell membrane in the liver [19].

In the present study, the rise in SGPT, SGOT, ALP, total bilirubin, direct bilirubin, cholesterol, and triglyceride levels induced by D-GalN administration was significantly reduced by pretreatment with MEFI (200 mg/kg and 400 mg/kg) suggesting that its hepatoprotective activity might be due to its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes.

Histopathological Studies in D-GalN induced hepatotoxicity

The hepatoprotective effect was confirmed by histological examination of the liver tissue of control and treated animals. The histological profile of the control animals showed normal hepatic architecture with distinct hepatic cells well-preserved cytoplasm sinusoidal spaces and a central vein. The histological architecture of D-GalN treated liver section showed massive fatty changes and the loss of cellular boundaries. However, administration of MEFI at higher doses significantly normalized these defects in the histological architecture of the liver. The phytoconstituents like flavonoids and tannins showed excellent protection to liver architecture almost to the level of the Silymarin-treated groups, showing its potent hepatoprotective effects in animal models (**Figure 9**).



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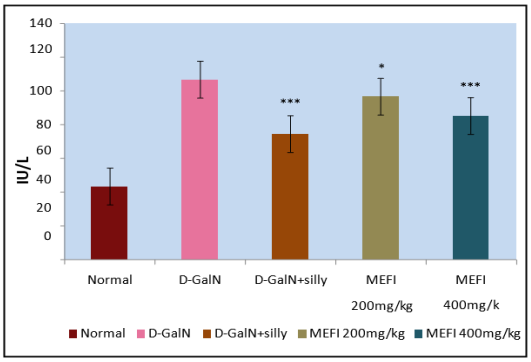

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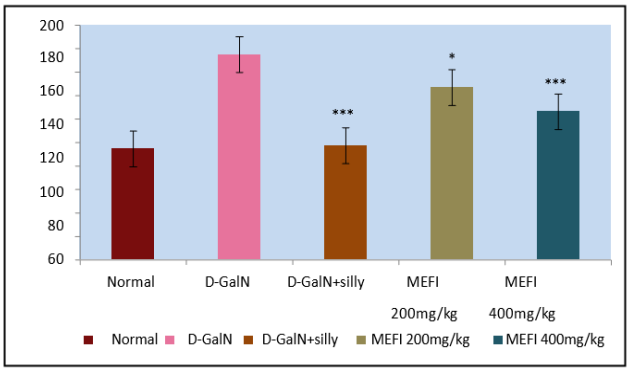

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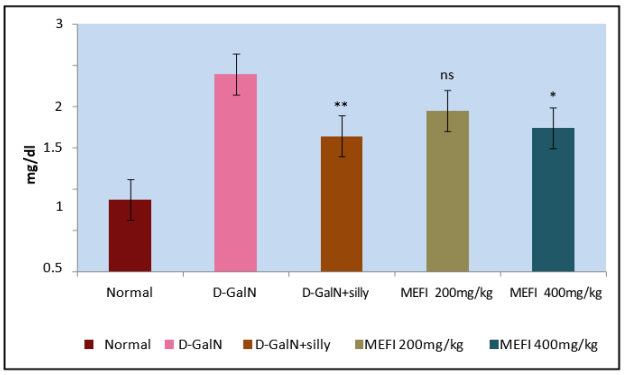

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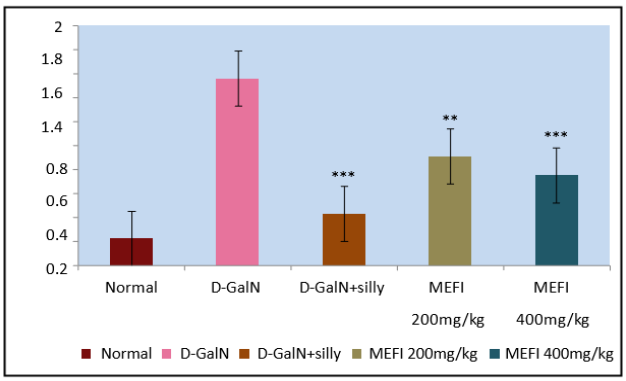

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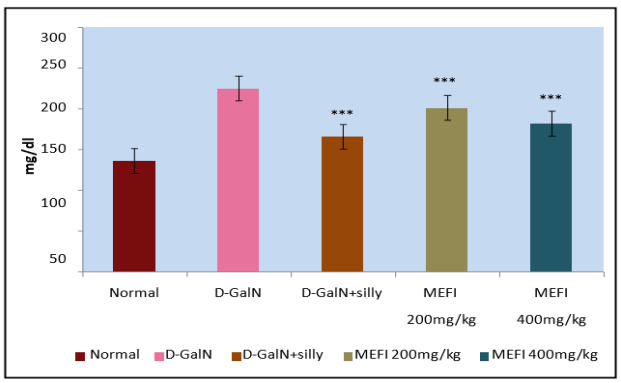

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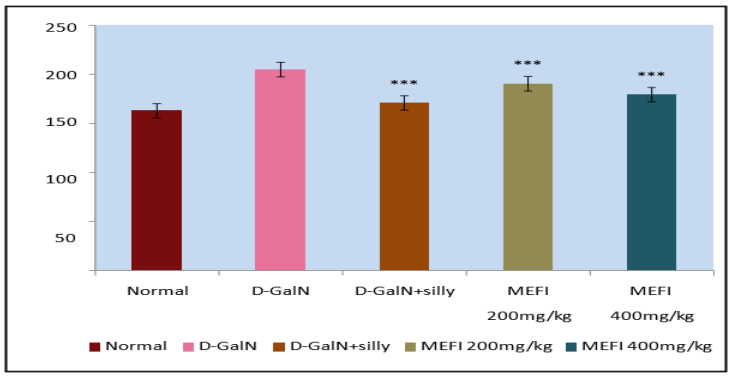
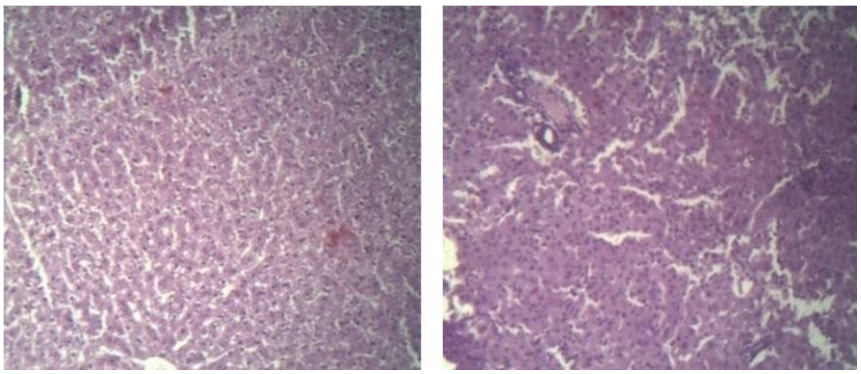
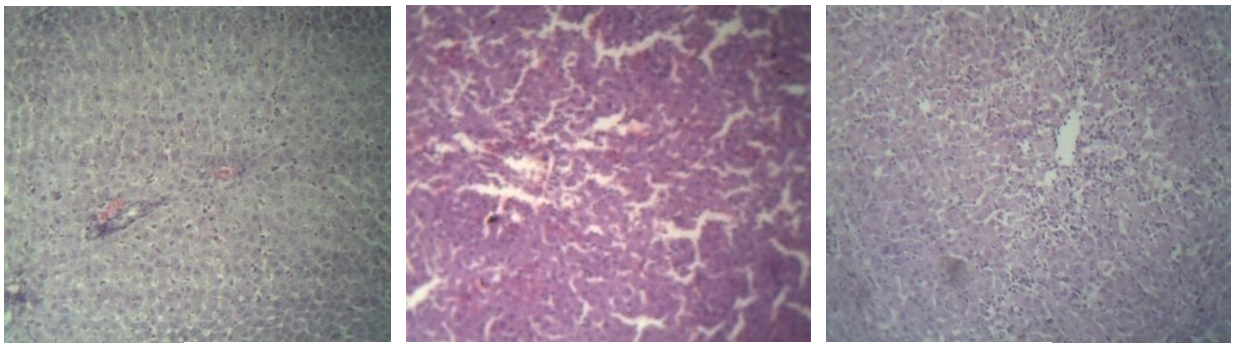


(7)

Fig. 2. Effect of MEFI on SGPT levels in D-GalN induced hepatotoxicity;
Fig. 3. Effect of MEFI on SGOT levels in D-GalN induced hepatotoxicity; **Fig. 4.** Effect of MEFI on ALP levels in D-GalN induced hepatotoxicity; **Fig. 5.** Effect of MEFI on levels of total bilirubin in D-GalN induced hepatotoxicity; **Fig. 6.** Effect of MEFI on levels of direct bilirubin in D-GalN induced hepatotoxicity;
Fig. 7. Effect of MEFI on levels of cholesterol in D-GalN induced hepatotoxicity;
Fig. 8. Effect of MEFI on levels of triglyceride in D-GalN induced hepatotoxicity



(a) (b)



(c) (d) (e)

Fig. 9. Photograph of liver architecture in D-GalN induced hepatotoxicity in rats (a). Liver architecture of normal control (b). Liver architecture of D-GalN treatment (c). Liver architecture of D-GalN treatment + 50 mg/kg Silymarin treatment (d). Liver architecture of D-GalN treatment + 200 mg/kg of 70% MEFI (e). The liver architecture of D-GalN treatment + 400 mg/kg of 70% MEFI

CONCLUSION

The 70% methanolic extract of *Fagonia indica* Burm. revealed significant hepatoprotective activity in a dose-dependent manner by reducing the elevated level of biochemical enzyme when treated with D-GalN which may be due to the presence of flavonoids and tannin. Further research is sought to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

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