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Hepatoprotective activity of the methanolic extract of *Fagonia indica* Burm in carbon tetra chloride induced hepatotoxicity in albino rats

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ABSTRACT

Objective: To investigate hepatoprotective activity of the methanolic extract of *Fagonia indica* Burm. on CCl_4 induced hepatotoxicity in albino rats. **Methods:** Animals in Group 1 served as vehicle control, Group 2 served as hepatotoxin (CCl_4 2ml/kg, s.c) treated group, Group 3 served as standard (Silymarin 50mg/kg, *p.o.*) treated group. Group4 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 marker like SGOT, SGPT, ALP, Bilirubin (Total & Direct) and Cholesterol. The histopathological studies also show the hepatic protection of the test extracts. **Results:** The levels of the biochemical parameters such as SGPT, SGOT, ALP, Total bilirubin, Direct bilirubin and Cholesterol were significantly increased in CCl_4 treated rats when compared with the normal group ($P<0.05$), but the MEFI (400 mg/kg, bw) treated rats showed maximum reduction of SGOT (114.83±1.51), SGPT (164.33±1.25), ALP (154.83 ±1.53), Total bilirubin (1.55±0.01), Direct bilirubin (0.65±0.009) and Cholesterol (193.00±1.06) in a significant manner. Histopathological studies also reveal the hepatoprotection property of MEFI in a dose dependent manner. **Conclusions:** These results suggest that MEFI in different doses showed significant hepatoprotective activity against CCl_4 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.

1. Introduction

The liver is a vital organ of human body which performs detoxification of the exogenous xenobiotic, drugs, viral infection and chronic alcoholism. The liver is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Liver diseases have become a worldwide problem and are associated with significant morbidity and mortality. The principal causative factors for the liver diseases in developed countries are excessive alcohol consumption, and viral-induced chronic liver diseases while in the 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 (CCL_4), thioacetamide (TAA), etc.)[2].

In liver disorders the ability of natural antioxidant system is impaired. Free radicals are generated in cells by environmental factors such as ultraviolet radiation, pollutants, x-rays, as well as by normal metabolism. Free radicals induce an oxidative state that can lead to cellular membrane injury with the consequent alteration in metabolic processes. Reactive oxygen species (ROS) plays an important role in the pathogenesis of various degenerative human diseases and have been implicated in atherosclerosis, liver disorders, lung and kidney damage, aging and diabetes mellitus [3].

Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India [5]. *Fagonia indica* Burm. is one such green plant which is abundantly grown & used as important medicinal plant [6]. However much of its medicinal importance is not assessed. By literature survey it is found that the

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leaves of the plant contain main 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-Dglucopyranoside. It also contains triterpenoid, saponins, alkaloids, coumarins, flavonoids and tannins [6]. *Fagonia indica* Burm.has been proven for analgesic and anti-microbial activity [7], as anti tumor activity [8], as anti diabetic activity [9], for the treatment of ear infection [10], and anti protozoal and cytotoxic activities [11]. This plant contains flavonoids hence we have planned to study its hepatoprotective property. Free radicals cause organ toxicities which are well reported. Therefore, there is a possibility that the plant may have a protective role. Keeping this in view it was thought that the plant *Fagonia indica* Burm. which is abundantly grown & used as medicinal plant may have a protective role in hepatic toxicities induced by carbon tetrachloride.

2. Materials and methods

2.1. Plant collection and identification:

The plant was collected from Mandvi, Kutch. and plant material was identified and authentified by Dr. P. S. Nagar, Professor, Faculty of Science, Department of Botany, The Maharaja Sayajirao University, Baroda, Gujarat, India. A specimen has been preserved in the department of the Botany of the University.(Specimen no. SDPC/2011-12/06)

2.2.Extraction preparation

In the present work, the authenticated shade dried plant *Fagonia indica* Burm, approximately (500 g), were powdered to coarse particle size no. (#) 40 and subjected to extraction with70% methanol in a soxhlet extractor for 48 h. The total methanol extract was filtered and concentrated to dryness at 40 ℃ [12].

2.3. Chemicals

Carbon tetrachloride (CCl_4) collected from Shree Dhanvantary Pharmacy College, Kim, Surat. Silymarinwere purchased from Microlab Pvt. Ltd., Banglore.

2.4. Acute toxicity (LD_{50}) studies

The acute toxicity for 70% MEFI was determined on albino female rat, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose method of OECD guideline No.420 given by CPCSEA was adopted for toxicity studies.

2.5. Animals

Healthy albino rats (150-250gm, 12-14 weeks age) were housed in cages with free access to standard rat chow diet and water ad libitum and acclimatized to the surroundings for one week prior to the experiment. Animals were harbored on a light/dark cycle (12/12 hr) at a constant temperature (25 ℃±3 ℃) and relative humidity (50±20%). The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Reg. no. 1103/PO/abc/07/CPCSEA).

2.6. Hepatoprotective activity

In the dose response experiment, albino rats were randomly assigned into 5 groups of 6 individuals each.
Group-I:Animals (-ve control) were administered 1ml distill water p.o., for 5 days.
Group-II: Animals (+ve control) were administered 1ml distill water p.o., for 5 days.
Group-III:Animals were administered with silymarin50 mg/kg p.o., for 5 days.
Group-IV: Animals were administered with 70% methanolic extract 200 mg/kg p.o., for 5 days.
Group-V: Animals were administered with 70% methanolic extract 400 mg/kg p.o., for 5 days.
Group-I received olive oil (1ml/kg) s.c., on 2nd and 3rd day.
Group-II, III, IV and V received CCl_4: olive oil (1:1) at a dose of 2ml/kg s.c., on 2nd and 3rd 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 6th day under mild ether anesthesia. 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 [13]. The liver samples were dissected out, blotted off blood, washed with saline and also stored it in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically [14].
The results are shown in Table No.1

2.7. Histopathology

Small pieces of liver tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope for the analyzing the altered architecture of the liver tissue due to CCl_4 challenge and improved liver architecture due to pretreatment with test extracts and standard drug [14]. The results are shown in Figure-1.

2.8. Statistical analysis

Results were expressed as Mean±SEM, (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. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01

Table 1

Effect of MEFI on enzyme SGOT, SGPT, ALP, Total bilirubin, Direct bilirubin, and Cholesterol levels in blood serum of CCl_4 induced hepatotoxicity.

Groups	Treatment	SGPT IU/L	SGOT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl	Cholesterol 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
GroupII	CCl_4	235.16±1.57	144.33±1.99	195.16±2.02	2.61±0.01	1.70±0.01	214.5±1.66
GroupIII	Silymarin	146.33±1.83***	93.83±1.55***	140.5±1.47***	1.47±0.16***	0.67±0.01***	172.66±1.22***
GroupIV	MEFI 200mg/kg	177.66±1.89***	122.33±1.20***	167.66±1.54***	1.84±0.03***	0.82±0.007***	204.33±0.88***
GroupV	MEFI 400mg/kg	164.33±1.25***	114.83±1.51***	154.83±1.53***	1.55±0.01***	0.65±0.009***	193.00±1.06***

Each value represents Mean±SEM, (n=6). Values in the parentheses indicate '*P*' value.*$P<0.05$; **$P<0.01$; ***$P<0.001$; ns= not significant; compared to CCl_4 group. One way ANOVA followed by Dunnett.'s multiple comparison tests.

and ***<0.001, when compared with control and toxicant group as applicable.

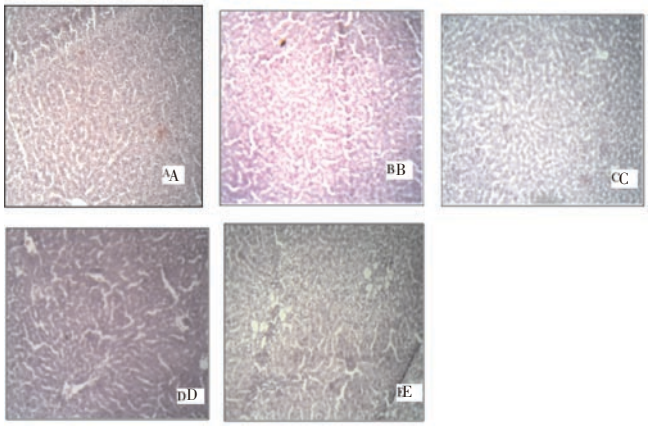

Figure 1: Photograph of liver architecture in CCl_4 induced hepatotoxicity in rat, Fig. A (Liver architecture of Normal), Fig. B (Liver architecture of CCl_4 treatment), Fig. C (Liver architecture CCl_4 treatment + 50 mg/kg Silymarin treatment), Fig. D (Liver architecture CCl_4 treatment + 200 mg/kg of MEFI), Fig. E (Liver architecture CCl_4 treatment + 400 mg/kg of MEFI).

3. Results

3.1. Acute toxicity (LD_{50}) studies

An attempt was made to identify LD50 of MEFI. Acute toxicity studies were carried out according tofixed dose method of OECD guideline No.420. Hence, no mortality was observed at 2000 mg/kg in rat. It was thought that 2000 mg/kg was the cut off dose. Therefore 1/10th and1/5th (200 mg/kg, 400 mg/kg) dose was taken as effective dose for all further In-vivo studies.

3.2. Hepatoprotective activity

A significant increase ($P<0.05$) in serum SGPT, SGOT, ALP, Total bilirubin, Direct bilirubin and Cholesterol levels was observed in animals treated with CCl_4 (2ml/kg s.c.) as compared to normal. Pretreatment with MEFI (200 mg/kg and 400 mg/kg p.o.) for 5 d decreases the above parameters significantly ($P<0.05$) as compared to CCl_4 treated group. Silymarin pretreatment produced significant decrease (p<0.05) in the above parameter when compared to CCl_4 treated group (Table No. 1).

3.3. Histopathological studies

Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus whereas CCl_4 treated group showed various degree of fatty degeneration like ballooning of hepatocytes, infiltration of lymphocytes and the loss of cellular boundaries. Administration of MEFI at higher doses (400 mg/kg, p.o.) significantly normalized these defects in the histological architecture of the liver (Figure 1).

4. Discussions

Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective activity. The rise in serum levels of SGPT, SGOT, ALP, Total bilirubin, Direct bilirubin and Cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. Carbon tetrachloride induces hepatotoxicity by metabolic activation therefore it selectively causes toxicity in liver cells maintaining semi normal metabolic functions. CCl_4 metabolically activated by CYP450 dependent mixed oxidase in the endoplasmic reticulum to form a trichloromethyl free radical ($CCl^{\bullet}3$), which combined with cellular lipids and proteins in presence of oxygen to induce lipid peroxidation [15]. Highly reactive tricolor free radicals formation directly attacks the poly unsaturated fatty acids of the endoplasmic reticulum and thus cause over production of SGOT, SGPT, ALP, Total bilirubin, Direct bilirubin and Cholesterol [16].

These result in change in structure of endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6 phosphatase activation, leading to liver damage [17].

Hepatotoxic compounds such as CCl_4 are known to cause marked elevation in serum transaminase. In the present study, pre-treatment with MEFI (200 mg/kg and 400 mg/kg) attenuated the increases in the activities of SGOT, SGPT, ALP, Total bilirubin, Direct bilirubin and Cholesterol was

found to be lower than the CCl_4 treated group indicated that MEFI (200 mg/kg and 400 mg/kg) protects the CCl_4 induced hepatic damage.

Silymarin is a known hepatoprotective compound obtained from Silybum marianum is reported to have a protective effect on plasma membrane of hepatocytes and possess multiple mechanisms of actions against different hepatotoxic agents. The antioxidant property and cell regenerating functions as a result of increased protein synthesis were considered as most important actions.Antioxidant potential of MEFI is believed to be due to the presence of flavonoids and tannins that regenerate the hepatocytes and stabilize the membrane by scavenging free radicals. The study shows that MEFI at higher dose (400 mg/kg p.o.) is comparable with standard drug Silymarin. The histopathological findings reveals that the phytoconstituents like flavonoids and tannins which are present in the plant extract showed excellent protection to liver architecture almost to the level of the Silymarin treated groups, showing its potent hepatoprotective effects in animal model.

Thus, the extract also reveals the significant hepatoprotective activity in a dose dependent manner by the reducing the elevated level of biochemical enzyme when they are treated with CCl_4. Hence 70% MEFI proven hepatoprotective activity may be due to presence of flavonoids and tannin. Further research is sought to explore the exact mechanism of action and phytoconstituents responsible for the pharmacological response.

Conflict of interest statement

We declare that we have no conflict of interest.

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