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Hepatoprotective activity of aqueous and ethanol extracts of *Feronia elephantum* Correa. Stem bark and Root

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ABSTRACT

In the present study, the hepatoprotective activity of aqueous and ethanol extracts of *Feronia elephantum* Correa. (Rutaceae) stem bark and root was evaluated against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. The CCl_4 (5 ml/kg)-intoxicated rats were enhanced the serum transaminase (serum glutamic oxaloacetic transaminase [SGOT] and serum glutamic pyruvic transaminase [SGPT]), alkaline phosphatase (ALP), total bilirubin (TB), total cholesterol (TC), while total protein (TP) level was reduced. Treatment with aqueous and ethanol extracts of stem bark and root of *F. elephantum* (50 and 100 mg/kg) has brought back the altered levels of biochemical markers of hepatic damage such as SGOT, SGPT, ALP, TB, TC, and TP to the near normal levels in the dose-dependent manner as well as histopathological examination was carried out to evaluate hepatoprotection. The results of this study strongly indicate that *F. elephantum* has a potent hepatoprotective action against CCl_4-induced hepatic damage in rats.

Key words: Carbon tetrachloride, Feronia elephantum, hepatoprotective, LIV 52

INTRODUCTION

The liver is an organ of paramount importance, due to its unique and considerable regenerative capacity, even a moderate cell injury is not reflected by a measurable change in its metabolic functions. The etiology of the liver disorders depends on various factors such as nutritional, biochemical, bacteriological, viral, or environmental aberration. The liver plays a significant role not only in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly but also in the metabolism of fats, carbohydrates, proteins, and immunomodulation.[1] Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects, and relatively low cost.[2] In the absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief.[3] Attempts are being made globally to get scientific evidence for these traditionally reported herbal drugs. *Feronia elephantum* (Rutaceae) is small- to medium-sized deciduous tree with a straight trunk and more or less oval or rounded crown, bark whitish to pale, or dark gray.[4] It is used to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching, and snake and insect poisoning, while the stem bark and root were prescribed for biliousness in various traditional systems of medicine.[5] The plant has been reported various pharmacological activities including hypoglycemic, hypolipidemic,[6] antiulcer,[7] and anti-diabetic[8] activities. The literature review indicated that the hepatoprotective activity of stem bark and root of *F. elephantum* has not been clinically evaluated so far. In view of this, the present study was aimed to evaluate the hepatoprotective activity of the stem bark and root of *F. elephantum* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats and to prove its claim in the folklore practices against liver disorders.

MATERIALS AND METHODS

Plant Material

The stem bark and root of *F. elephantum* were collected from the Sultanpur, Uttar Pradesh, in October 2009. The plant was authenticated at the National Botanical Research Institute, Lucknow, Uttar Pradesh, and the voucher specimen (NBRI/CIF/123/2009) was deposited in the Department of Pharmacognosy, Technocrats Institute of Technology, Pharmacy, Bhopal, Madhya Pradesh.

Chemicals and Reagents

All the chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine chemicals Ltd., Mumbai, India. The standard drug LIV 52 was obtained as gift sample from Himalaya Drug Company, India. Standard kits for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), and bilirubin were obtained from Span Diagnostics Ltd., India.

Extraction

The powdered stem bark (500 g) and root (200 g) were evenly packed in the Soxhlet apparatus and extract with various solvent increasing polarity, namely, petroleum ether, chloroform, ethyl

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acetate, and ethanol about 26 h, separately. The aqueous extraction was carried out by cold maceration process. The solvents were removed by vacuum distillation, and the concentrated extracts were packed in airtight container for the further studies.

Phytochemical Analysis

Aqueous extracts of stem bark (ASFE), ethanol extracts of stem bark (ESFE), aqueous extracts of root (ARFE), and ethanol extracts of root (ERFE) were subjected to identify the presence of various phytoconstituents, namely alkaloids (Dragendroff's test), steroids and terpenoids (Leibermann Burchard test), tannin and phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), and amino acids (Ninhydrin test) by usual methods prescribed in standard texts.[9,10]

Animals

Wistar albino rats and mice of either sex were used for the study of the crude extracts. The Institutional Animal Ethics Committee has approved the project (Registration No. TIT/IAEC/831/P'cog/2010/02). The animals were kept at 27°C ± 2°C, relative humidity 44–56%, and light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with water *ad libitum* and standard diet (Lipton, India), and the food was withdrawn 18–24 h before the start of the experiment. All the experiments were performed in the morning according to the current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Acute Toxicity Study

Acute toxicity study was performed according to the OECD guidelines. Swiss albino mice were used for acute toxicity study, and the animal was kept fasting for overnight providing water *ad libitum*, after which the extracts were administered orally 2000 mg/kg and observed the mortality 3 of 3 animals. Then, dose level was reduced to 300 mg/kg and mortality was observed in 1 of 3 animals. The same dose administrated again to confirm the toxic dose. The animals were safe up to 500 mg/kg.

Hepatoprotective Activity

The rats were randomly divided into various groups of six rats in each (n = 6).[11-13]

Group I (normal): 0.5% sodium carboxymethyl cellulose (0.2 ml/100 g, p.o.) once daily for 7 days.

Group II (toxic control): 0.5% sodium carboxymethyl cellulose (0.2 ml/100 g, p.o.) once daily for 7 days and single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Group III (standard): LIV 52 (400 mg/kg, po) once daily for 7 days and a single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Group IV and V: ASFE (50 and 100 mg/kg, po) once daily for 7 days and single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Group VI and VII: ESFE (50 and 100 mg/kg, respectively, orally) once daily for 7 days and a single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Group VIII and IX: AREF (50 and 100 mg/kg, respectively, orally) once daily for 7 days and a single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Group X and XI: ERFE (50 and 100 mg/kg, respectively, orally) once daily for 7 days and a single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on the 7th day.

Assessment of Hepatotoxicity

On the 8th day, the animals were anesthetized with light ether anesthesia, and the blood was obtained from animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. The serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters including SGOT and SGPT,[14] ALP,[15] serum bilirubin,[16] serum protein,[17] and serum cholesterol.[18]

Histopathology Study

A portion of the liver tissue of all the animal groups was excised and was then washed with normal saline. The liver tissues were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and were then processed for paraffin embedding. Using a microtome, sections of 5 mm thickness were taken and stained with hematoxylin and eosin. These sections were examined under the light microscope using a magnification of ×100.[19]

Statistical Significance

The results of the study were expressed as a mean ± standard error of the mean, n = 6. ANOVA was used to analyze and compare the data, followed by Dunnett's test for multiple comparison shown in (Table 1).

The elevated SGOT and SGPT levels found in CCl_4-treated rats were 112.86 and 117.16, respectively. ASFE, ESFE, ARFE, and ERFE (50 and 100 mg/kg) were decreased the levels of SGOT and SGPT when compared with toxic group. ASFE and ESFE showed more significant activity than the AREF and ERFE. The level of ALP in the normal control group was found to be 68.60, and elevated value was found to be 131.50 on CCl_4-treated rats. ASFE and ARFE (50 and 100 mg/kg) were decreased the level of ALP when compared with ESFE and ERFE. The level of total bilirubin (TB) in normal control group was found to be 0.90 and in CCl_4-treated rats was found to be 4.58. The 50 and 100 mg/kg of ESFE have decreased the level of TB to 3.40 and 3.33, respectively. The level of total cholesterol (TC) in normal control group was 68.89, and elevated level was found to be CCl_4-treated rats (146.33). ASFE, ESFE, ARFE, and ERFE were decreased the levels of TC when compared with toxic group. The decrease the level of total protein (TP) was found to be 2.10 in CCl_4-treated rats, where the level of TP in normal rat group was found to be 6.47. ASFE, ESFE, ARFE, and ERFE (50 and 100 mg/kg) significantly increase the level of TP when compared with normal control group rats.

Histopathological Examination

The normal liver tissue showed normal architecture, but the rats were treated with CCl_4 showed extensive signs of necrosis, fatty changes, and hydropic changes, while the rats were treated with LIV 52 showed no alteration in the normal architecture of liver. ASFE and ESFE (50 and 100 mg/kg) showed micro fatty changes with a dense collection of lymphoid cells suggesting evidence of very little necrosis or degeneration, while ARFE and ERFE (50 and

100 mg/kg) showed micro fatty changes with focal collection of few lymphocytes surrounding the central vein prominent Kupffer cells [Figure 1].

DISCUSSION

CCl_4-induced hepatotoxicity is a commonly used model for the screening of hepatoprotective activity, which is characterized the system of the xenobiotic-induced hepatotoxicity.[20,21] When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream, and their estimation serum is a useful quantitative marker of the hepatocellular damage.[22]

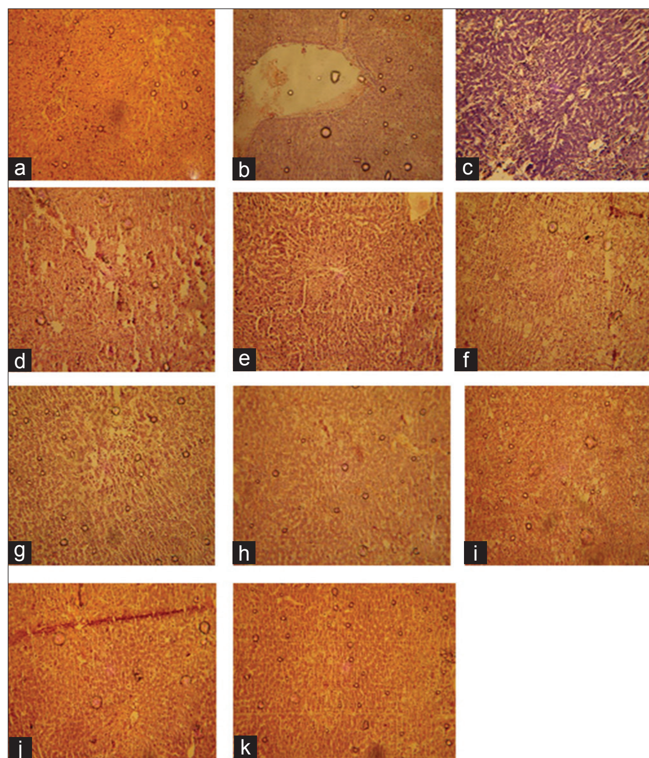

Figure 1: Photomicrographs of liver sections (a. Normal liver tissue; b. Intoxicated with carbon tetrachloride [CCl_4]; c. LIV 52 and CCl_4; d. Aqueous extracts of stem bark [ASFE] 50 mg/kg and CCl_4; e. ASFE 100 mg/kg and CCl_4; f. Ethanol extracts of stem bark [ESFE] 50 mg/kg and CCl_4; g. ESFE 100 mg/kg and CCl_4; h. Aqueous ethanol extracts of root [ARFE] 50 mg/kg and CCl_4; i. ARFE 100 mg/kg and CCl_4; j. Ethanol extracts of root [ERFE] 50 mg/kg and CCl_4; and k. ERFE 100 mg/kg and CCl_4)

The increased levels of SGOT, SGPT, ALP, TB, and TC were found in CCl_4-treated rats due to its severity of hepatocellular damage by the liver cell destruction or changes in the membrane permeability. The rise in SGOT activity is almost always due to hepatocellular damage and is usually accompanied by the rise in SGPT.[23] An increase in ALP reflects the pathological alterations in the biliary flow. Pre-treatment with ASFE, ESFE, ARFE, and ERFE attenuated the increased activities of these enzymes in serum caused by CCl_4. Recovery toward the normalization suggests that these extracts caused parenchymal cell regeneration in the liver, thus protecting membrane fragility and thereby decreasing enzyme leakage.

Determination of TB represents an idea for the assessment of hepatic functions, and any abnormal increase in the levels of bilirubin in the serum indicates hepatobiliary disease and severe disturbance of hepatocellular function.[22] The extracts mediated suppression of the increased bilirubin level caused by CCl_4 suggests the possibility of the extracts being able to stabilize biliary dysfunction.

Hepatocellular damage increase in cholesterol levels was observed in CCl_4-induced rats, which may be due to the inability of the diseased liver to remove cholesterol from circulation.[23] ASFE, ESFE, ARFE, and ERFE (50 and 100mg/kg) caused a significant reversal of the altered TC levels toward the normal.

The decrease in the level of TP observed in CCl_4-treated rats may be associated with the decrease in the number of hepatocytes, which in turn may result in decreased hepatic capacity to synthesize protein. These extracts at both the doses caused a significant reversal of TP toward the standard, which indicating the increased hepatic capacity of the liver.

Histopathological study was performed to provide direct evidence of the hepatotoxicity of CCl_4 and the effect of the ASFE, ESFE, ARFE,

Table 1: Effect of ASFE, ESFE, ARFE, and ERFE on the SGOT, SGPT, ALP, TB, TC, and TP in CCl_4-induced hepatic damage in rats

Group/dose (mg/kg)	SGOT (IU/dl)	SGPT (IU/dl)	ALP (IU/dl)	TB (mg/dl)	TC (mg/dl)	TP (g/dl)
0.5% sodium CMC (0.2 ml/100 g)	29.34±0.26	28.54±0.23	68.60±0.23	0.9±0.02	60.89±0.31	6.47±0.12
50%CCl_4 (5 ml/kg))	112.86±0.18	117.16±0.24	131.50±0.31	4.58±0.08	146.33±0.30	2.10±0.03
LIV 52 (400)	40.33±0.16**	39.16±0.26**	77.15±0.27**	2.16±0.06**	70.60±0.19**	4.56±0.17**
ASFE (50)	49.10±0.20**	48.67±0.31**	88.43±0.22**	3.10±0.09**	95.87±0.13**	3.5±0.11**
ASFE (100)	46.10±0.18**	42.50±0.27**	78.45±0.19**	2.80±0.12**	90.43±0.23**	3.96±0.08**
ESFE (50)	53.67±0.19**	52.19±0.13**	95.33±0.29**	3.40±0.16**	107.50±0.28**	3.20±0.25**
ESFE (100)	52.10±0.20**	50.67±0.37**	93.45±0.53**	3.32±0.10**	99.40±0.19**	3.29±0.14**
ARFE (50)	48.76±0.22**	47.39±0.25**	91.12±0.34**	3.27±0.07**	98.24±0.22**	3.48±0.07**
ARFE (100)	47.56±0.33**	47.34±0.26**	90.76±0.28**	3.23±0.10**	94.89±0.23**	3.67±0.03**
ERFE (50)	56.78±0.21**	58.33±0.3**	99.50±0.17**	3.76±0.09**	107.85±0.11**	3.90±0.14**
ERFE (100)	55.87±0.14**	57.40±0.24**	98.52±0.15**	3.60±0.12**	105.33±0.29**	3.12±0.07**

Values are mean±SEM, *n*=6. One-way ANOVA followed by Dunnett's multiple compression test. **Denotes statistically significance of *P*<0.01 compared with toxic control group and for TP compormal group. SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, TC: Total cholesterol, TP: Total protein, ASFE: Aqueous extracts of stem bark, ESFE: Ethanol extracts of stem bark, ARFE: Aqueous ethanol extracts of root, ERFE: Ethanol extracts of root, SEM: Standard error of the mean

and ERFE. The test samples and LIV 52 reversed the hepatic lesions to some extent which indicated the hepatoprotective activity.

The preliminary phytochemical screening of ASFE, ESFE, ARFE, and ERFE revealed that the presence of various phytoconstituents including glycosides, flavonoids, saponins, alkaloids, triterpenoids, and phenols. The phytochemicals such as flavonoids,[24,25] triterpenoids, and alkaloids[26] were reported as hepatoprotective substance induced by various chemicals. Hence, these above phytoconstituents may be responsible for hepatoprotective activity of ASFE, ESFE, ARFE, and ERFE.

Future Prospects

The biochemical and histological evidence show that the pre-treatment with the ASFE, ESFE, ARFE, and ERFE protected rats against CCl_4-induced hepatotoxicity. It provides a scientific support for the traditional use of *F. elephantum* in liver disorders. Further studies should be conducted to determine the active compounds that are responsible for the hepatoprotective effects and the mechanism of action involved in this.

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