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Research Article

THE HEPATOPROTECTIVE ACTIVITY OF PHARMACOGNOSTICALLY AUTHENTICATED *FERONIA ELEPHANTUM* CORREA. STEM BARK AND ROOT

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ABSTRACT

F*eronia elephantum* Correa. (Rutaceae) is a popular medicinal plant in various Indian systems of medicine and its stem bark and root were used as anti-inflammatory, anti-ulcer, anti-bacterial, antifungal agents and in the treatment of liver diseases. In view of its medicinal importance and taxonomic confusion the pharmacognostic studies were carried out while it includes the hepatoprotective activity of aqueous and ethanol extracts of *F. elephantum* stem bark (ASFE & ESFE) and root (ARFE & ERFE) were evaluated against carbon tetrachloride (CCl_4) induced hepatic damage in rats. The pharmacognostic studies provided referential information for identification of this crude drug. The results of hepatoprotective study strongly indicate that *F. elephantum* have potent hepatoprotective action against CCl_4 induced hepatic damage in rats. The biochemical and histological evidences show that the pretreatment with aqueous and ethanol extracts of *F. elephantum* stem bark and root effectively protected rats against CCl_4 induced hepatotoxicity. It provides a support for the traditional use of *Feronia elephantum* in liver disorders.

Key words: *Carbon tetrachloride, Feronia elephantum, Hepatoprotective, Pharmacognostic studies.*

INTRODUCTION

Liver is a largest organ of the body comprising 2-3% of the total adult body weight and also the central site for the biotransformation of xenobiotic chemicals. It is involved in the detoxifying mechanism of the body. Liver is responsible for detoxifing the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury. The liver damage caused by pathogens as well as chemical agents is of similar nature[1]. At present, in spite of an increasing need for agents to protect the liver from damage, modern medicine lacks a reliable liver protective drug. Therefore, a number of natural substances have been studied to evaluate the hepatoprotective activity[2].

Ethanopharmacological survey conducted among herbal practitioner of Dharauli, Sultanpur, Uttar Pradesh, the stem bark and root of *F. elephantum* were used to treat various liver diseases. The plant *F. 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 grey[3]. The plant have been reported various pharmacological activities including hypolipidemic[4], antiulcer[5] and anti-diabetic[6]. The present study was to carried out pharmaconostic studies and hepatoprotective activity of the stem bark and root of *F. elephantum* against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant materials

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

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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 (Himalaya Drug Company, India) was purchased from chemist. Standard kits for SGOT, SGPT, ALP and bilirubin were obtained from Span Diagnostics Ltd., India.

METHODS

PHARMACOGNOSTIC STUDIES

Morphological and microscopical studies
The gross morphological character of the stem bark and root were described based on the shape, size, colour, surface, fracture and appearance of cut surface[7]. For microscopical examination, the paraffin embedded specimens were sectioned using a Rotary Microtome at a thickness of 10-12 µm. Dewaxing of sections was made using routine procedure[8] and the sections were stained with Toluidine blue method[9]. Powdered material of root was cleared with sodium hydroxide and mounted in glycerin medium after staining with phloroglucinol and hydrochloric acid.

Instruments used
Photographs of different magnifications were taken with Nikon Labphot2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background[10].

Physicochemical analysis
The dried powdered material of stem bark and root were subjected to physicochemical analysis including total ash, water soluble ash, acid insoluble ash, alcohol soluble and water soluble extractive values to determine the quality and purity of the plant material[11].

Preliminary phytochemical screening
The stem bark and root were dried and powdered with a mechanical grinder and pass through sieve no 45. The dried powdered material (250 g) was extracted by successively with petroleum ether, chloroform, ethyl acetate, ethanol by using soxhlet apparatus and water by cold maceration, separately. These extracts were filtered and concentrated by a rotary vacuum evaporator and kept in a vacuum dessicator for complete removal of solvent. The presence of various phytoconstituents viz. alkaloids (Dragendroffs test), steroids (Leibermann Burchard test), flavonoids (Shinoda test), amino acids (Ninhydrin test), etc. were detected by usual methods prescribed in standard texts[12].

PHARMACOLOGICAL STUDIES

Animals
All the experiments were carried out using male, Swiss albino mice (25-30 g) and Wistar rats of either sex (180-200 g) procured from animal house of IHB(Institutional of Health and Biologicals), Mhow, Madhya Pradesh. The animals had free access to food and water, and they were housed in a natural (12 h each) light-dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of the laboratory animals was taken as per the CPCSEA regulations (Reg. No: TIT/IAEC/831/ P'cog/2010/02).

Acute toxicity study
Acute toxicity study was carried out using Swiss albino mice (25-30 g). The animals were fasted 12 h before the experiment and were administered with single dose of ASFE, ESFE, ARFE and ERFE and observed for mortality up to 48 h. On the basis of toxicity, the dose of next animal was determined as per OECD guideline 420.

Hepatoprotective activity
The rats either sex were randomly divided into various groups of six rats in each (n = 6)[13].

Group I (Normal): 0.5% sodium carboxy methyl cellulose (0.2 ml/100g, po) once daily for 7 days.

Group II (Toxic control): 0.5% sodium carboxy methyl cellulose (0.2 ml/100g, po) once daily for 7 days and single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on 7^{th} day.

Group III (Standard): Liv 52 (400 mg/kg, po) once daily for 7 days and single dose of 50% CCl_4 with olive oil (5 ml/kg, ip) on 7^{th} 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 7^{th} day.

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

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

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

Assessment of hepatotoxicity

On the 8th day the animals were anaesthetized with light ether anaesthesia and the blood (0.4-0.6 ml/per animal) 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 & SGPT, ALP, serum bilirubin, serum protein and serum cholesterol[14].

Histopathology study

A portion of the liver tissue of all the animal groups was excised and 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 then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness were taken and stained with hematoxylin and eosin[15]. These sections were examined under light microscope using a magnification of 100X.

Statistical Significance

The results of the study were expressed as mean ± SEM, n = 6. ANOVA was used to analyze and compare the data, followed by Dunnet's test for multiple comparisons.

RESULTS

The dried and coarse powdered of stem barks and roots of *F. elephantum* were extracted with the solvents of increasing polarity successively by soxhlet apparatus, while the aqueous extract was obtained by cold maceration method. The percentage yield of stem bark and root extracts (viz. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous) were presented in table (Table 1). The percentage yield of aqueous extract of stem bark was greater than aqueous extract of root.

The values obtained from the physicochemical analysis such as loss on drying, ash value and extractive value are useful for selection and authentification of *F. elephantum* for explore their traditional uses and the values were presented in table (Table 2).

The preliminary phytochemical screening of various extracts of stem bark and root were showed the presence of various phytoconstituents and the results presented in table (Table 3).

The morphological characters of stem bark and root (Figure 1) were described based on the shape, size, colour, surface, fracture and appearance of cut surface and reports are presented in table (Table 4).

The stem bark consists of 2 zones such as: outer zone (periderm) and inner zone (secondary phloem) (Figure 2). Periderm is 350 µm thick, fissured at several places forming wide, shallow irregular fissures. The phloem cells are compressed into thick leands without recognition of any cellular detail. The periderms exfoliate in the form of thick flacks. Secondary phloem is the major part of the bark. It is differentiated into outer collapsed phloem and inner non collapsed phloem. Collapsed phloem is characterized by presence of large, irregular masses branchy sclereids. The brachy sclerieids are elongated and cylindrical. The walls are thick and lignified. The cell lumen is narrow. The walls have narrow canal like pits. Inner to the scleried zone is seen dilated phloem rays alternating with narrow radial bands of collapsed phloem elements and thick tangential blocks of phloem fibers. Further in the inner zone, the phloem rays become narrow and straight comprising narrow elongated compactly arranged thin walled cells. The sclerenchyma bands are in regular, tangential cylinders transversed by the phloem rays. In between and alternating with the fiber

cylinders occur crushed and collapsed sieve elements. Next to the collapsed phloem is a narrow reason of non collapsed phloem, where the sieve element and parenchyma cells are intact. The sieve elements are rectangular in sexional outline and are arranged in radial files. The companion cells are located outer lateral sides of the siev elements. The phloem sclerenchyma (fibers) are seen is small descrete masses, the phloem rays are narrow. Calcium oxalate prismatic crystals are seen in the phloem ray cells. Calcium oxalate crystals are also seen in abedence associated with the blocks of phloem fibers. The crystals are predominentely prismatic type.

The powder of the stem bark powder exhibits the bundle of fibers and crystal strands. The thick vertical bundles of fibers are seen in abundant. These bundles are always associated with the prismatic crystals. The crystals are in regular vertical fills or strands. The crystals are double pyramidal, rhomboidal and aboidal in shape. Branchy scleroides are isodiamrtic scleroides called branchyscleroids are abundant in the powder. These cells are cubical in shape; they have thick lignified walls with simple pits. The lumen is wide and empty. Scattered in the powder are also calcium oxalate prismatic crystals varying shape and size (Figure 3).

The thick root is 2.2 mm in diameter. It consists of wider periderm which peals of and rolls back at certain places. Inner thin periderm occurs crushed dark cylinder of cortex followed ley wide secondary phloem (Figure 4). Secondary phloem consists of wide, dilated funnel shaped rays comprising tangentially rectangular thin walled cells and conical radial segments of phloem tissue. The conical segments possess tangential bloens of fibers alternating with tangential, dark, trust phloem elements. Secondary xylem cylinder is 2.2 mm thick. It is solid and dense. It consists of prominent straight xylem rays and circular, wide solitary diffusely distributed vessels. The diameters of the vessels are increase from center periphery. The narrow vessels are 30 µm in diameter and the wider vessels are 100 µm in diameter.

The root powder consists of fibers, parenchyma cells, vessels, starch grains and calcium oxalate (Figure 5). The fibers are abundant in the powder. They are seen in broken pieces as well as inter act entire form. Some of the fibers are narrowing lumened with thick walls and without pits. Other type of fibers has wide lumen thin walls and well developed pits. The narrow fibers are up to 700µm long and wide fibers are 450µm longs. Parenchymatus cells are elongated, scale like thin walled parenchyma cells and cells various shapes and sizes with dark contents are often seen in the powder. Vessels elements are fairly wide cylindrical vessels elements are seen scattered into powder. They have wide, circular perforatious at the ends. The lateral walls possess dense, multi-seriate bordered pits. The vessels elements are 180-220µm long. The prismatic crystals are common in the parenchymatous ground cells. The starch grain are circular concentric with central hylum. The grains are upto 40 µm wide. The crystals are 20×50µm in size.

There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioural changes at a dose level of 500 mg/kg. This finding suggests that ASFE, ESFE, ARFE and ERFE were safe in or non-toxic to rats up to 500 mg/kg. Hence, the doses of 50 and 100 mg/kg were selected for the hepatoprotective study.

In this study, the protective effect ASFE, ESFE, ARFE and ERFE on CCl_4 induced hepatotoxicity was evaluated through various biochemical parameters and the results were presented in table (Table 5).

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 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 TB in normal control group was found to be 0.90 and CCl_4 treated rats was found to be 4.58. The 50 and 100 mg/kg of ESFE were decreased the level of TB to 3.40 and 3.33, respectively. The level of 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 TP was found to be CCl_4 treated rats (2.10), 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.

The normal liver tissue showed normal architecture but the rats were treated with CCl_4 showed extensive sings of necrosis, fatty changes and hydropic changes. 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. 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 6).

DISCUSSION

Morphological, microscopical and powder characters of the stem bark and root of *F. elephantum* gives more valuable information on plant material for further studies as well as to explore their traditional uses.

From the study aqueous and ethanol extracts of stem bark of *Feronia elephantum* (50 and 100 mg/kg) showed more significant activity than the root extracts, in which the dose level of 100 mg/kg showed potent hepatoprotective activity than the dose level of 50 mg/kg. The hepatic damage induced by CCl_4 is well known to be mediated by its free radical metabolites such as $CCl^{\cdot}_3$ and CCl_3COO-, which leads to auto oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane. Hepatocellular necrosis leads to very high level of SGOT, SGPT and ALP released from liver in the blood[2]. ASFE, ESFE, ARFE and ERFE (50 and 100 mg/kg) treated groups showed reduction in the levels of SGOT, SGPT and ALP towards the

respective normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4.

The biochemical and histological evidences show that the pretreatment with ASFE, ESFE, ARFE and ERFE (50 and 100 mg/kg) effectively protected rats against CCl_4 induced hepatotoxicity. It provides a support for the traditional use of *Feronia 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.

Table 1: Percentage yield of various extracts of stem bark and root of *Feronia elephantum*

Name of the extracts	% yield (w/w)	
	Stem bark	Root
Pet. Ether	8.05	6
Chloroform	6	10
Ethyl acetate	10	12
Ethanol	10	10
Aqueous	16	14

Table 2: Physicochemical analysis of stem bark and root of *Feronia elephantum*

Parameters	Stem bark (% w/w)	Root (% w/w)
Loss on drying	5.55	10.52
Total ash	not more than 16.67	not more than 20
Acid insoluble ash	not more than 49.94	not more than 33.27
Water soluble ash	not more than 66.61	not more than 66.61
Water soluble	not less than 8	not less than 16
Alcohol soluble	not less than 16	not less than 16

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Table 3: Preliminary phytochemical tests of various extracts of stem bark and root of *Feronia elephantum*

Extracts / Phytoconstituents	Pet. ether		Chloroform		Ethyl acetate		Ethanol		Aqueous	
	A	B	A	B	A	B	A	B	A	B
Alkaloids	+	+	+	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	-
Sponins	-	-	+	-	-	-	-	-	+	+
Flavonoids	-	-	+	-	+	+	+	+	+	+
Steroids	+	+	-	-	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-	-	-	-	-

A=Bark,Root,
B=Stem,
+ = Positive,
-= Negative

Table 4: Macroscopical characters of stem bark and root of *Feronia elephantum*

Characters	Stem bark	Root
colour	Black	Pale brown
odour	Mild aroma	Mild aroma
taste	Bitter	Tasteless
shape	More or less oval; or rounded	Straight
size	1.2 to 3.5 cm long and 0.5 to 2.25 cm wide	Long and 5 to12.5 cm diameter
surface	Rough fissured	Roughish plain
Texture	Fibrous	Fibrous

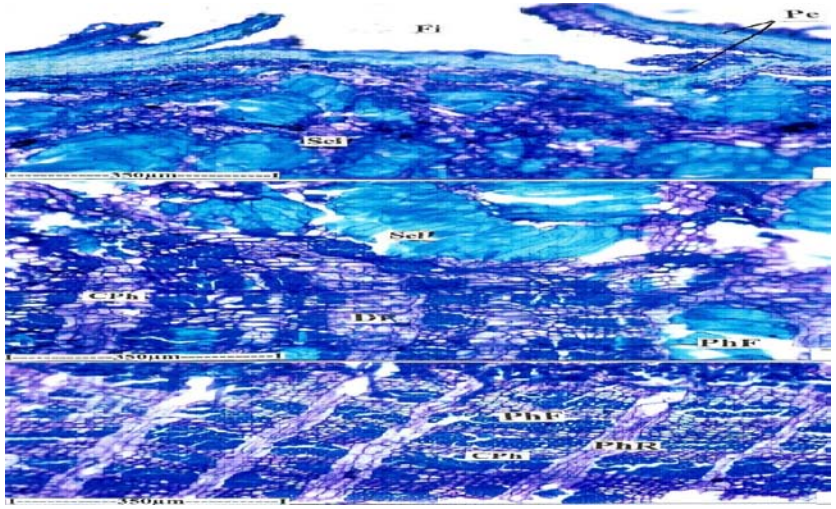
Table 5: Hepatoprotective activity of ethanol and aqueous extracts of stem bark and root of *Feronia elephantum*

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/100g)	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 compared with normal group.

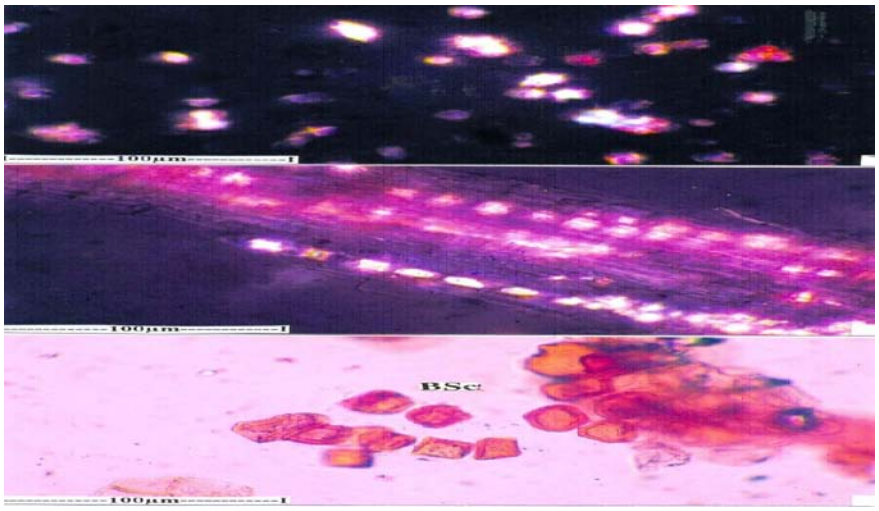
Figure 1: Stem bark and root of *Feronia elephantum*

Figure 2: Microscopical characters (TS) of stem bark of *Feronia elephantum*



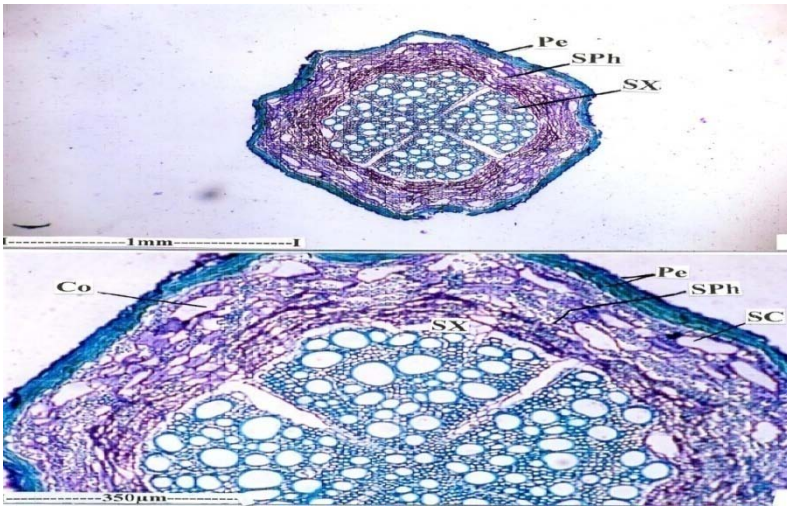
[Fi- Fiber; Pe- Periderm; Scl- Scleroids; Cph- Collapsed phloem; DR- Dilated ray; PhF- Phloem fiber; PhR- Phloem rays]

Figure 3: Powder microscopical characters of stem bark of *Feronia elephantum*



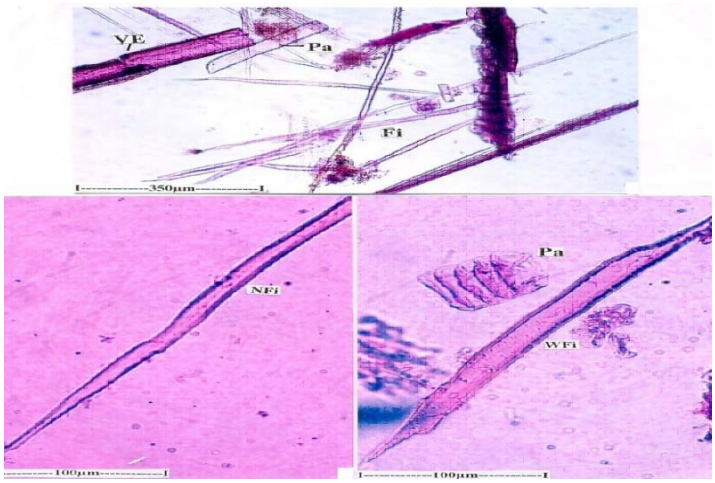
[Bsc- Branchy scleroides]

Figure 4: Microscopical characters (TS) of root of *Feronia elephantum*



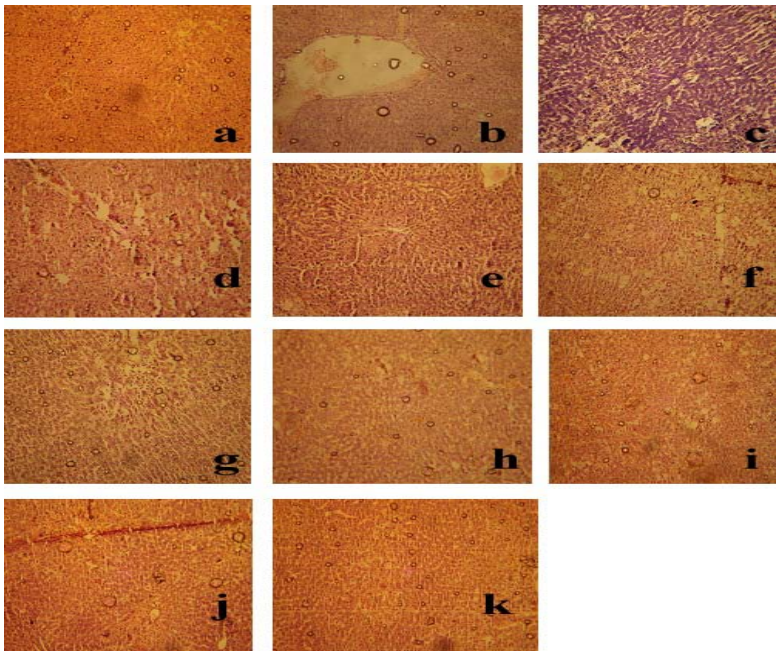
[Pe- Periderm; SPh- Secondary phloem;SX- Secondary xylem;SC- Secretory cavity; Co- Cortex]

Figure 5: Powder microscopical characters of root of *Feronia elephantum*



[VE- Vessels element; Pa- Parenchyma; Fi- Fiber; NFi- Narrow fiber; WFi- Wide fiber]

Figure 6: Photomicrographs of liver sections



(a. Normal liver tissue; b. Intoxicated with CCl_4; c. LIV 52 and CCl_4; d. ASFE 50 mg/kg andCCl_4; e. ASFE 100 mg/kg andCCl_4; f. ESFE 50 mg/kg and CCl_4; g. ESFE 100 mg/kg and CCl_4; h. ARFE 50 mg/kg and CCl_4; i. ARFE 100 mg/kg and CCl_4; j. ERFE 50 mg/kg and CCl_4; k. ERFE 100 mg/kg and CCl_4)

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