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Antihepatotoxic activity of *Phyllanthus fraternus*

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Different fractions of alcoholic extracts of aerial parts and roots of *Phyllanthus fraternus* Webster (Euphorbiaceae) were screened for antihepatotoxic activity on carbon tetrachloride induced liver damage in albino rats. The degree of protection was measured using biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), total protein (TP) and total albumin (TA). The methanol fraction was found to be the most active, which was further supported by a significant recovery of hepatocytes in the histopathological study of the liver showing almost complete normalization of the tissues as neither the fatty accumulation nor the necrosis was observed.

1. Introduction

About 600 commercial preparations, mainly plant crude extracts, with claimed liver protecting activity are available all over the world. About 100 Indian medicinal plants belonging to 40 families are used in the herbal formulations [1], which are used for the treatment of various diseases of the liver. *Phyllanthus fraternus* Webster (Euphorbiaceae) is an herb wildly distributed in the northern region of India and is used as a folklore remedy for the treatment of various diseases of liver by the traditional healers and tribals [2].

The basic and distinguishing features of *P. fraternus* [3], *P. nirurii* and *P. amarus* are: *P. fraternus* Webster is an annual herb, the stem is non-erect and 30 cm tall, leafy shoot is 5–10 cm long, oblong and joined to the branchlets of the stem, six sepals in the flower, distributed in India, Pakistan and introduced into Saudi Arabia, Africa and West Indies [4]; *P. nirurii* L. is an erect annual herb, leafy shoot is less than 5 cm long, acute and terete, flower bears 6 tepals in two whorls of three each, distributed in Tropical Asia from India to Malaysia [5]; *P. amarus S*chum & Thonn is an erect annual herb, 30 cm tall, leafy shoot is less than 5 cm, elliptic, joined to the branchlets of the stem, 4–5 sepals in the flower, distributed in India and Yemen [5]. The plant is seen in the rainy seasons during the months of September and October in marshy areas.

A literature survey has revealed that neither the systematic assessment of antihepatotoxic activity, nor the phytochemical investigation of the plant have been done so far. We have, therefore, conducted a thorough pharmacological screening for the antihepatotoxic activity of different fractions of alcoholic extract of aerial parts and root extract of the plant on carbon tetrachloride induced liver damage in rats. The study showed different degrees of activity on measuring the different biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), total protein (TP) and total albumin (TA). The histopathological study also exhibited a significant recovery of the hepatocytes in different sections of the liver, wherein the methanolic fraction showed almost complete normalization of the tissues. Other fractions including the root extract also decreased the enzymes levels significantly. Total protein was increased while total albumin was decreased by all fractions in different proportions.

2. Investigations, results and discussion

As shown in Table, activities of liver enzymes SGPT, SGOT and total albumin were markedly elevated while total protein level was decreased in CCl_4 treated animals in comparison to normal values. Administration of different fractions of aerial parts and roots of *Phyllanthus fraternus* at a dose of 500 mg/kg body weight markedly prevented CCl_4 induced elevation of serums GPT, GOT and total albumin, and diminution of total protein.

The petroleum ether, acetone, methanol, root and total alcoholic extracts decreased SGPT by 84.6, 73.8, 40.4, 44.2 and 48.2 units/ml, SGOT by 100.4, 110.37, 64.4, 67.3 and 69.1 units/ml respectively. The methanol fraction was found to be the most active, even more than the standard drug silymarin at a comparable dose level (500 mg), exhibiting a decrease of 40.4 units/ml in SGPT and

Table: Effect of various extracts of *Phyllanthus fraternus* on serum enzymes, total proteins and albumin in CCl_4 induced liver damage in rats[a]

Group No.	Treatment	Dose	SGPT[a] Units/ml	SGOT[a] (Units/ml)	TP g/dl[a]	TA g/dl[a]
1	Normal/ Control	Nil	22.1 ± 0.98	8.8 ± 0.75	1.1099 ± 0.02	0.2741 ± 0.26
2	CCl_4 intoxicated control	0.5ml/kg, (p.o.)	151.7 ± 2.32	130.6 ± 1.73	1.395 ± 0.04	0.1456 ± 0.05
3	Petroleum ether fraction	500 mg/kg, (p.o.)	84.6 ± 1.54**	100.4 ± 2.05*	1.1757 ± 0.0002*	0.2700 ± 0.15
4	Acetone fraction	500 mg/kg, (p.o.)	73.8 ± 0.14*	110.3 ± 1.32*	1.2616 ± 0.02**	0.2679 ± 0.04***
5	Methanol fraction	500 mg/kg (p.o.)	40.4 ± 1.55*	64.4 ± 0.23*	1.1546 ± 0.0004*	0.2638 ± 0.05
6	Root extract	500 mg/kg (p.o.)	44.2 ± 1.55*	67.3 ± 0.91*	1.1642 ± 0.02*	0.2367 ± 0.01
7	Silymarin (Standard)	500 mg/kg (p.o.)	67.4 ± 0.9*	96.4 ± 1.23*	1.1609 ± 0.09***	0.293 ± 0.33
8	Total alcoholic extract	500 mg/kg (p.o.)	48.2 ± 0.36*	69.1 ± 0.45*	1.1319 ± 0.07**	0.2456 ± 0.08

[a] SGPT: Serum glutamic Pyruvate transaminase, SGOT: Serum glutamic oxaloacetic transaminase, TP: Total protein, TA: Total albumin; p.o: Per oral; * $P < 0.001$; ** $P < 0.01$; *** $P < 0.1$ Vs intoxicated control using Student's t-test.
Values are mean ± S. E of eight rats.

64.4 units/ml in SGOT as compared to silymarin (67.4 and 96.4 units/ml) against intoxicated control (151.7 and 130.6 units/ml) in comparison to normal values (22.1 and 8.8 units/ml, respectively). The root extract was also found to possess a significant activity as compared to other fractions of the aerial parts. Total protein was increased while total albumin was decreased by all fractions in different proportions, wherein methanol (TP: 1.1546 g/dl; TA: 0.2638 g/dl) and root extract (TP: 1.1642 g/dl; TA: 0.2367 g/dl) were found to be most active as compared to standard drug silymarin (TP: 1.1609 g/dl; TA: 0.2933 g/dl) against CCl_4 intoxicated control (TP: 1.3959 g/dl; TA: 0.1456 g/dl) in comparison to normal values (TP: 1.1099 g/dl and TA: 0.2741 g/dl respectively) (Table).

Histopathological studies of the liver were also carried out, which showed swelling and necrosis in hepatocytes in CCl_4 treated rats in comparison to normal control rats. Administration of different extracts of the plant exhibited a significant recovery of hepatocytes in different sections of the liver, wherein the methanolic fraction showed almost complete normalization of the tissues. The fatty accumulation and necrosis were not observed. The acetone fraction also exhibited a significant recovery of the liver tissues. Other fractions also showed a considerable recovery of the liver tissues.

It has been, therefore, suggested that the different extracts/fractions of *Phyllanthus fraternus* have varied degrees of antihepatotoxic activity. The methanol fraction of alcoholic extract of aerial parts and root extract possessed antihepatotoxic activity even better than the standard drug silymarin. Further, the extracts of the plant did not produce any gross behavioral change or mortality even at a dose of 500 mg/kg, p.o. in rats. It can, therefore, be said that the plant extract is non-toxic and may be used as a safe drug.

The preliminary phytochemical investigation has shown the presence of triterpenes and phenolic components in the aerial parts and root of the plant. It was, therefore, concluded that either the concentration of the active principle is much higher or the most active component is present in the methanolic fraction and root extract. Other fractions also contain the active compounds either in comparatively lower concentration or some other less active principles.

3. Experimental

3.1. Plant material

The aerial parts and root of *Phyllanthus fraternus* were collected from the campus of Hamdard University, New Delhi in the months of September and October 1998, and identified by a taxonomist, Department of Botany. A voucher specimen no. 11274 has been preserved in the herbarium of Jamia Hamdard for future reference.

3.2. Preparation of the plant extracts

The dried aerial parts (6.0 kg) were exhaustively extracted with ethanol in a soxhlet apparatus. The concentrated viscous crude alcoholic extract (1.5 kg) was dissolved in boiling methanol and kept at room temperature, solidified fats (0.3 kg) were removed by suction, and the filtrate so obtained was concentrated to get a viscous mass and then subsequently fractionated into petroleum ether (60–80 °C) (0.2 kg), acetone (0.25 kg) and methanol soluble (0.3 kg) fractions. The roots (100.0 g) were also extracted with ethanol and concentrated to get a viscous solid (10.0 g). After evaporating off the solvents, the above fractions and the total alcoholic extract (0.4 kg) were prepared with gum acacia (1 : 1) for oral administration to albino rats.

3.3. Animals

The study was carried out on Wistar albino rats (150–200 g) of either sex as reported in the literature [6]. The rats were bred in a colony in the Central Animal House of Jamia Hamdard. They were fed with a standard pellet diet (Gold Mohar, Lipton India Ltd., Calcutta) and water *ad libitum*. Before and during the experiment, the rats were kept under standard environmental conditions (temp. 25–28 °C and 12 h light/dark cycle). Eight animals in each group were used in all sets of experiments.

3.4. Testing of antihepatotoxic activity

The animals were divided into seven groups of eight rats in each and were treated as follows: Group- 1 (normal control without any treatment); Group-2 (intoxicated control) was given 0.5 ml CCl_4/kg body weight in liquid paraffin (1 : 1) by oral route for 15 days. Groups 3, 4, 5, 6 and 8 received 0.5 ml CCl_4/kg in liquid paraffin (1 : 1) followed by oral treatment with petroleum ether, acetone, methanol, root and total alcoholic extracts/fractions (500 mg/kg each) respectively for 15 days; whereas group-7 was given silymarin as standard dose (500 mg/kg) for 15 days.

After the treatment blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30 °C for 15 min and analyzed for various biochemical parameters.

3.5. Assessment of the liver function

The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) were estimated by a reported method [7]. The total protein and total albumin were also measured according to the reported methods [8, 9].

3.6. Statistical analysis

The results of the biochemical estimations are reported as mean ± S.E. Total variation, present in a set of data was estimated by one way analysis of variance (ANOVA). Student's test and Dennett's test were used for determining the significance [10, 11].

3.7. Histopathological studies of the liver

The histopathological studies were carried out by a reported method [12]. The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the livers were removed and washed with normal saline. Small pieces of liver tissues were processed and embedded in paraffin wax. Sections of 5–6 microns in thickness were cut, stained with haematoxylin and eosin, and then studied under an electron microscope.

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References

1 Handa, S. S.; Sharma, A.; Chakarborti, K. K.: Fitoterapia **57**, 307 (1986)
2 Kirtikar, K. R.; Basu, B. D.: Indian Medicinal Plants, 2. Ed. Vol. 1, p. 2225, Lalit Mohan Babu and Co., Allahabad 1975
3 Mitra, R. L.; Jain, S. K.: Bulletin of Botanical Survey of India, p. 161, 271 (1985)
4 Abedin, S.; Mossa, J. S.; Al-Said, M. S.; Al-Yahya, M. A.; in: Chaudhury, S. A. (Ed.): Flora of Kingdom of Saudi Arabia, p. 298, National Agriculture and Water Research Centre, Riyadh, Saudi Arabia 2001
5 Wood, J. R. I.: A Handbook of the Yemen Flora, p. 185, Royal Botanical Garden, Kew, England 1997
6 Handa, S. S.; Singh, A.: J. Ethnopharmacol. **49**, 119 (1995)
7 Reitman, S.; Frankel, S.: Am. J. Clin. Pathol. **28**, 56 (1957)
8 Wooton, I. D. P.: Microanalysis in medical biochemistry, 4. Ed. p. 138, J. and A. Churchill Ltd., London 1964
9 Dumas, B. T.; Watson, W. A.; Biggs, H. G.: Clin. Chim. Acta. **31**, 87 (1971)
10 Woolson, R. F.: Statistical Methods for the Analysis of Biomedical Data, John Wiley and Sons Inc, New York 1987
11 Dennett, C. W.: Biometrics **20**, 482 (1964)
12 Luna, L. G.: Manual of histology: Staining methods of armed force institute of pathology, 3. Ed. McGraw-Hill, Book Co., New York 1968

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