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Diuretic activity of the roots of *Flacourtia indica*

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Abstract

Plan:*The present study was carried out to investigate the diuretic activity of the ethanolic extract of roots of Flacourtia indica (Flacourtiaceae).*

Prologue*: It is a popular drug used to treat many diseases in Indian traditional medicine. In order to substantiate the traditional uses of the drug the scientific study of diuretic activity was undertaken by us.*

Methodology*: Air dried; powdered roots of Flacourtia indica were defatted with petroleum ether first and subsequently extracted with 90% alcohol. The preliminary phytochemical screening of the ethanolic extract revealed the presence of carbohydrates, tannins, phenolic compounds and terpenoids. The diuretic activity of ethanolic extract was evaluated by determining the urine volume and electrolyte concentration in albino rats. Frusemide (10 mg/kg) was used as standard while normal saline (0.9%) was used as control. Ethanolic extract of the drug (250 mg/kg and 500mg/kg) were used as tests.*

Outcome: *The ethanolic extract (500 mg/kg) showed significant increase in urine volume as well as Na^+, K^+ and Cl^- ion concentration in albino rats.*

Keywords**:** *Flacourtia indica* roots, ethanolic extract, acute toxicity, diuretic activity

1.Introduction

Flacourtia indica (Burm.f.) Merr. (Flacourtiaceae) is a plant that has been mentioned in traditional literature for treatment of jaundice[17, 18]. The roots are sweet, refrigerant, depurative alexipharmic and diuretic. They are useful in vitiated condition of 'pitta' and useful in poisonous bites, skin diseases, pruritus, erysipelas, strangury, nephropathy and psychopathy. The leaves are useful in pruritus and scabies.[1] This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera. [2, 3] A study on the phenolic composition of *F.indica*, showed that its pulp contained total phenolics, flavonoids and condensed tannins 334 µg (Gallic acid equivalents), 41 µg catechin/g and 1.4%condensed tannins [4].

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The protective effects of *F.indica* aerial parts extract against paracetamol-induced hepatotoxicity in rats has also been reported.[5] Three antimalarial compounds, pyrocatechol, homaloside D and poliothrysoside have been isolated from the aerial parts of *F.indica*[6] The *in vitro* antioxidant activity of methanolic and aqueous extract of *F.indica* leaves have also been reported. [7] The antihistaminic activity has been studied by using ethanolic extract of leaves of *F.indica* on experimental models.[8] It has been observed that the species *F.ramontchi* is very useful plant in treating inflammation and infectious diseases. [9]

2. Materials and Methods

2.1. Plant material

The Roots of *F.indica* were collected from Thiruvananthapuram, India and authenticated by taxonomists from the Pharmacognosy Division, Government Ayurveda Research Centre, Thiruvananthapuram, India (Herbarium Voucher specimen no. KUBH-5794).

2.2. Preparation of extract

500 g of air dried powdered roots of *F.indica* were defatted with one litre of petroleum ether and extracted with 90% alcohol in a continuous soxhlet extractor. The yield of the greenish brown extract (FIEE) was 4.05% w/w.[10]

2.3. Preliminary Phytochemical screening

Preliminary photochemical screening of the extract was done to determine the presence of various phytochemicals.[11]

2.4. Acute toxicity study

2.4.1. Animals

Male Albino rats (Wistar strain) weighing 150-200 g, procured from animal house of Medical College, Trivandrum, were used for the study. Animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed except during experimentation.

Acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development OECD) guidelines for Testing of Chemicals number 420[12]. The study was initiated with a sighting study aimed to determine the dose for the acute toxicity study as per the guidelines of IAEC (No: 04/07/2011/MCT).

The sighting study comprised of female rats dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. Since no information was available regarding the acute toxicity of the test substance, it was started with a dose of 300 mg/kg.

The rat was then observed for toxic effect for the first 30 min followed by hourly for 8h for the first 24 h. There were no signs of toxic effect or mortality observed on the rat within the 24 hours, then another rat with the next dose (2000mg/kg) and a similar procedure was carried out.Based on this observation main test was designed. Main test comprises of 5 female rats (including the previous one) which were chosen as it is the most sensitive gender to see the effect of treatment.[12]

2.4.2. Evaluation of diuretic activity

The animals were divided into four groups. Group-I, the normal control, received only saline solution. Group-II, the positive control, received frusemide at a dose of 500 mg/kg. Group-III and Group-IV received the alcohol extract, at doses of 250 and 500mg/kg, respectively. [13, 14, 15, 16]

2.4.3. Experimental design

Animals were deprived of food and water 18 h before the experiment. They were hydrated with 5ml/kg of water prior to drug/extract administration. Immediately after dosing, animals were placed in metabolic cages (2 in one cage), specially designed to separate urine and faeces. The urine was collected in measuring cylinder up to 5 h after dosing. The study was initiated as per the guidelines of IAEC (No: 04/07/2011/MCT).

During this period, animals were deprived of food and water. The parameters measured were total urine volume, urine concentration of Na^+,K^+ and Cl^- ions. Concentration of Na^+ and K^+ were determined using Flame photometer while Cl^- concentration was estimated titrimetrically using 0.02N $AgNO_3$ with 5% FIEE using potassium chromate as indicator. Appearance of brick red precipitate was taken as the end point.

2.5.Statistical analysis

SPSS (version 13.0) statistical program was used to carry out one-way analysis of variance (ANOVA) on the data, followed by Dunnett's test. Values are expressed as mean ± SEM of six samples. $P < 0.01$ was considered as significant.

Table 1. Diuretic activity of ethanolic extract of roots of *F. Indica* in albino rats

Group	*Urine volume (ml/100gm/hr)*	*Diuretic index*
Control	4.35 ± 0.13	-
Frusemide	7.32 ± 0.18	1.68
FIEE 250mg/kg	5.61± 0.13	1.29
FIEE 500mg/kg	7.13± 0.13	1.64*

FIEE = *Flacourtia Indica* Ethanolic Extract; n=3, *p value <0.001

Table 2. Na^+, K^+ and Cl^- ion concentrations in the urine samples of albino rats

Group	*Na^+ (meq./L)*	*K^+ (meq./L)*	*Cl^- (meq./L)*
Control	53.16±1.08	17.40±2.71	56±1.07
Frusemide	9 1. 61±2.1	2 9. 54±3.05	101±1.98
FIEE 250mg/kg	60.00±2.32	25.23± 2.43*	68±2.13
FIEE 500mg/kg	86.61±1.78*	27.56± 2.5*	89±2.22*

FIEE = *Flacourtia Indica* Ethanolic Extract; n=3, *p value <0.001

3. Results and Discussion

Preliminary Phytochemical screening

Phytochemical screening of the alcoholic extract showed the presence of carbohydrates, tannins, phenolic compounds and terpenoids.

Acute toxicity study

The results of acute toxicity studies showed that the extract was not toxic upto2000mg/kg.

Evaluation of diuretic activity

The results of diuretic activity are shown in Tables 1 and 2. The extract shows significant diuretic activity at dose of 500mg/kg. The activity may be attributed to the polyphenolic constituents. The results corroborate its use in traditional medicine as a diuretic. Further studies are needed to develop formulations of the drug.

4. Conclusion

The diuretic activity of *F.indica* may be due to the individual or combined action of bioactive constituents present in it. Further phytochemical and pharmacodynamic investigations are required to find the active constituents responsible for the activity and to understand the precise mechanism of diuretic exhibited by ethanolic extract of roots of *F.indica*.

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