Available online at: www.cbioresearch.com/journal.html ISSN No. 0973-8207

Evaluation of Diuretic Activity of Hydroalcholic Extract of *Artocarpus Heterophyllus* Leaves in Rats

Sunil Koshy[1], Prinkesh Fanasia[*], Prima Freeda D'souza[*], Gopikrishna[*], A.R.Shabaraya[*], Megha Patel[*], Sajo John[*] and V. A. J. Huxley[**]

[1]Department Of Pharmacology, Srinivas College Of Pharmacy, Valachil-Mangalore, Karnataka, India
[2] Biotech Research laboratory, Department of Zoology, Thiru. Vi. Ka. Govt. Arts College, Tiruvaraur – 610 003, Tamilnadu, India

Abstract

The present study was undertaken to investigate diuretic effect of hydro alcoholic extract of the Artocarpus heterophyllus leaves in albino rats. Acute oral toxicity study was performed as per OECD guidelines. In acute oral toxicity study, mortality was not observed up to 2000 mg/kg bodyweight. Hydro alcoholic extract of the Artocarpus heterophyllus leaves were administered at the doses of 200 and 400 mg/kg, p.o., Furosemide 20mg per kg body weight was used as positive control in study. The diuretic effect of the extract was evaluated by measuring urine volume, sodium and potassium content. The extracts showed a potent diuretic effect with increase in electrolyte concentration in urine, when compared with standard drug (furosemide) in albino rats. The present study provides a quantitative basis for explaining the folkloric use of Artocarpus heterophyllus as a diuretic agent.

Keywords: Herbal medicines, Diuretic activity, urine output,

Introduction

In recent years, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems (Dahanurkar et al., 2000). Medicinal plants constitute a source of both traditional and modern medicines. Herbal medicines derived from plant extract, are increasingly being utilized to treat a wide variety of clinical diseases, with relatively little knowledge of their modes of action (Begum et al., 2008). Diuretics play an important role in situations of fluid overload, like acute and chronic renal failure, hypercalciuria, cirrhosis of liver, and also as an antihypertensive agent. A number of diuretics like mannitol, thiazides, furosemide, and ethacrinic acid are used in practice. Drug-induced diuresis is beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemia (Agunu et al., 2005).

The jackfruit (***Artocarpus heterophyllus***) is a species of tree in the *Artocarpus* genus of the mulberry family (*Moraceae*). Jackfruit (*Artocarpus heterophyllus*) is one of the most significant trees in tropical home gardens and is a medium-size evergreen tree typically reaching 8–25 m (26–82 ft) in height that is easily recognized by its fruit. The plant is known to possess varied medicinal properties. The leaves are useful in fever, boils, wounds and skin diseases. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and constipating (Hemborn, 1996). The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent (Sato and Fujiwara, 1996). The ash of Jackfruit leaves is used in case of ulcers. The dried latex yields artostenone, convertible to artosterone, and a compound with marked androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings (Vaidya Gogte, 2000). The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhoea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property and its pith is said to be abortifacient. The plant is reported to possess antibacterial, anti-inflammatory, antidiabetic, antioxidant and immunomodulatory properties (Gupta and Tandon, 2004).

Journal of Basic and Applied Biology, Vol. 6, No.3&4, 2012, pp. 97-101

On the basis of the traditional use of the plant as a diuretic, the present study was carried out using hydro alcoholic extract of *Artocarpus heterophyllus* leaves in an experimental model, to substantiate the folklore claim.

Materials and Methods

Collection of Plant Material:

Artocarpus heterophyllus Leaves were collected from different places of mangalore.The plant was identified and authenticated by Mr. Gopikrishna, Asst. Prof.Srinivas College Pharmacy, Mangalore.

Drugs and Chemicals:

All the drugs, chemicals and reagents were procured from Himedia suppliers Mangalore, INDIA. All the chemicals were of an analytical grade.

Preparation of Extract

Fresh leaves of *Artocarpus heterophyllus* were separated from plant and allowed to dry in sunlight for 15days and then homogenized to get a coarse powder. Powder (250g) was extracted with Hydroalcholic mixture (ethanol-60% and water-40% in 6:4 proportions) at room temperature by hot percolation method (soxhlet apparatus). The filtrate was collected and concentrated on heating mantle at 45æ%c till a syrupy mass was obtained. The extract was again dried by rotary evaporator and kept under refrigeration. The percentage yield was found to be 4.56 with respect to the initial dried plant material [8].

Animals

Albino Rats (150-200g) of either sex will be procured from Indian Institute of Sciences. They will be maintained under standard conditions (temperature 22 ± 2°C, relative humidity 50±5% and 12 h light/dark cycle).The animals will be housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They will have free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Acute Toxicity Study

The acute toxicity of Hydroalcholic leaf extracts of plant ***Artocarpus heterophyllus*** was determined using **Swiss albino mice** as per OECD425 guideline, the animals were observed continuously for the behavioural changes for the first 2,4h and then observed for mortality if any, after 24h.

Preliminary Phytochemical Screening

The phytochemical examination of the Hydroalcholic extract of *Artocarpus heterophyllus* leaves was carried out .

The chemical group tests were performed are as follows:

1**. Test for alkaloids:**

a) **Dragendorff's test**: To 2 mg of the extract 5 ml of distilled water was added, and then 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff s reagent (potassium bismuth iodide) was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

b) **Hager's test**: To 2 mg of the extract taken in a test tube, a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate confirmed the presence of alkaloids.

c) **Mayer's test**: A few drops of the Mayer's reagent (potassium mercuric iodide) were treated with 2 mg of extract. Formation of white or pale yellow precipitate indicated the presence of alkaloids.

2. **Test for carbohydrates**:

a) **Benedict's test:** 0.5 ml of water extract, 5 ml of Benedict's solution was taken and boiled for 5 minutes. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

b) **Fehling's test:** To 2 ml of extract, 1 ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicated the presence of reducing sugar.

c**) Molisch's test:** In a test tube containing 2 ml of extract, 2 drops of freshly prepared 20% alcoholic solution of á- naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. If red-violet ring appeared, indicating the presence of carbohydrates which disappeared on the addition of excess of alkali.

d) **Barfoed's test:** To the extract solution, few drops of Barfoed's reagent were added, boiled on water bath. Formation of brick red precipitate indicated the presence of carbohydrates.

3. Test for flavonoids:

a) **Ferric chloride test**: Test solution with few drops of ferric chloride solution shows intense green colour.
b) **Zinc-Hydrochloric acid reduction test**: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red colour.

c) **Lead acetate solution test:** Test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

4. Test for proteins:

a) **Biuret's test:** To 1 ml of hot extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops 3% w/v copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

b) **Xanthoproteic test:** Test solution after treating with concentrated nitric acid and on boiling, gave yellow precipitate.

c**) Ninhydrin test:** Test solution when treated with Ninhydrin reagent turned to blue colour.

6. Test for saponins

a) Foam test: In a test tube containing about 5 ml of an extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

b) Haemolysis test: 2 ml each of 18% sodium chloride solution in two test tubes were taken. To one test tube distilled water was added and to the other 2 ml of extract. Few drops of blood were added to both the test tubes. Mixed and observed for haemolysis under microscope.

Diuretic Activity

Lipschitz test described by was employed for the assessment of diuretic activity. In this method wistar albino rats were divided into four groups of six animals each and kept in standardize environmental conditions. The animals were fasted for 24hrs prior to the experiment and water was given ad libitum during fasting. First group of animals (negative control) received normal saline (25ml/kg,p.o),second group(positive control) received furosemide (20mg/kg ,p.o) and third and fourth groups of animals simultaneously received (200 ,400 mg/kg,p.o) Hydroalcholic extract of *Artocarpus heterophyllus* leaves. Immediately after dosing, the animals were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature. The volume of urine collected was measured upto 5hrs, after dosing. During this period no water and food was made available to the animals. The urine volume was measured with graduated Measuring cylinder. The parameters taken for each individual rat were total urine volume, concentration of Na^+, K^+ and Cl^- in urine for assessment of diuretic activity. Na^+ and K^+ concentration was determined with Flame photometer, while Cl^- concentration was estimated by titrimetrically by silver nitrate solution using diphenylcarbazone as indicator (Kumarasamyraja et al., 2011). The mean urine volumes were determined and diuretic potency was assessed by comparison of urine excretion due to the extracts with respect to the standard drug furosemide.

Statistical analysis

All results are expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett comparison test. *p*-values were calculated against vehicle control groups and $p<0.05$ was considered significant.

Result and Discussion

The Preliminary Phytochemical analysis of the Hydroalcholic extract of *Artocarpus heterophyllus* leaves revealed the presence of carbohydrates, proteins, Alkaloids and flavonoids. The present study revealed that Hydroalcholic extract of *Artocarpus heterophyllus* leaves at 250 and 500 mg/kg caused a dose dependent increase of urinary water and electrolytes concentration in normal rats. The results of 500mg/kg treated group showed significant change in chloride ion and urine volume compared with control group. In the present study Hydroalcholic extract treated groups at different doses (250mg/kg and 500mg/kg) showed significant effect on urinary potassium ion concentration. On the above results, it can be concluded that the extract produces diuretic effect with increase in electrolyte concentration in urine. Further studies are necessary to identify and isolate the active constituents responsible for the diuretic activity. These findings may provide a lead for further

investigations of the overall pharmacological actions of *Artocarpus heterophyllus* in more appropriate model. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and constipating (Hemborn, 1996). The latex is useful in dysopia, ophthalmic disorders and pharyngitis and also used as antibacterial agent (Sato and Fujiwara, 1996). The ash of Jackfruit leaves is used in case of ulcers. The dried latex yields artostenone, convertible to artosterone, and a compound with marked androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings (Vaidya Gogte, 2000).

Conclusion

The results obtained in this study provide a quantitative basis to explain the traditional folkloric use of *Artocarpus heterophyllus* as a diuretic agent.

Phytochemical components of qualitative analysis	Hydroalcholic extract of Artocarpus heterophyllus
Proteins	+
Carbohydrates	+
alkaloids	+
Flavonoids	+
Triterpenoids	+
glucose	+
Fructose	+
Glycosides	+
deoxySugar	+
Saponins	+
monosaccharides	-
Tannins	+

Table-1 Effect of *Artocarpus heterophyllus* on urine volume and electrolyte concentration

GROUPS	Urine Volume (ml/min/animal)	Na^+ Excreted in urine (meq/5h/animal)	K^+ Excreted in urine (meq/5h/animal)	Na + /K + ratio	Cl^- Excreted in urine (meq/5h/animal)
Vehicle control	0.85±0.02	0.06±0.005	0.06± 0.003	1.25	0.12±0.003
Furosemide	6.58±0.22**	1.27±0.045**	0.33±0.003**	4.14	1.45±0.053**
Hydroalcoholic 200mg/kg	1.95±0.03*	0.34±0.007*	0.11±0.003*	2.67	0.31±0.012*
Hydroalcoholic 400mg/kg	3.32±0.13**	0.63±0.021**	0.24±0.013**	3.02	0.62±0.021**

**= $p < 0.01$ = very significant, *= $p < 0.05$ = significant, Number of animals (N) = 6, Values are expressed as mean ± SEM.

References

Dahanurkar S.A., Kulkarni R.A. and Rege R.A., Pharmacology of medicinal plants and natural products, Indian J. Pharmacol. 2000; 32: 81-118.

Begum N., Mayuren C., Balaji N., Chinnapa Reddy Y and Aravind Kumar K., Evaluation of Hepatoprotective activity of Aqueous extract of *curcuma longa* in carbon tetra chloride induced hepatotoxicity in Rats, Adv. Pharmacol. Toxicol. 2008; 9(3), 33-36.

Agunu A, Abdurahman EM, Andrew, Muhaman Z. Diuretic activity of the stem-bark extracts of *Steganotaenia araliacea* hoehst. J Ethnopharmcol 2005; 96:471-75.

Hemborn. P. P. Contact therapy practiced by Mundas Chotanagar (Bihar). *Ethanobotany.* 1996; 8: 36-9.

Sato. M. and S. Fujiwara. Flavones with Antibacterial activity against carcinogenic bacteria. J. *Ethnopharmacol.* 1996; 54(2-3): 171-76.

Vaidya Gogte, V. M., *Ayurvedic Pharmacology y and therapeutic use of medicinal plants,* (Swami Prakashananda Ayurvedic Research centre, Mumbai, 2000; 656-57.

Gupta. K. and N. Tandon, *Review on Indian Medicinal Plants,* (Indian Council of Medical Research, New Delhi, 2004; 182-200.

Kumarasamyraja D, Shankar M, Gowrishankar NL.Preliminary phytochemical and diuretic potential of methanolic extract of *Azima tetrecahntha lam*,leaf.International journal of pharmacy and industrial research 2011;1:275-78

Kane SR ,Apte VA,Todkar SS, Mohite SK . Diuretic and laxative activity of ethanolic extract and its fraction of *Euphoria thymifolia linn* .International Journal of chem. Tech Research2009;1:149-52.

* Author for correspondence