



## Research Article

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### EVALUATION OF DIURETIC ACTIVITY OF ETHANOLIC EXTRACT OF *ARTOCARPUS HETEROPHYLLUS* SEEDS IN ALBINO WISTAR RATS

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#### ABSTRACT

Hypertension is a common presentation of Cardiovascular Diseases, which are attributed as a cause of death world wide. Diuretics are among the main stay treatment for hypertension. The use of *Artocarpus heterophyllus* seeds as diuretics has been documented in Ayurvedic literature. This study was done to evaluate the diuretic activity of ethanolic extract of *Artocarpus heterophyllus* seeds on acute administration in rats. 24 Albino Wistar rats of either sex were taken and divided into 4 group of 6 animals each. Group I received normal saline (10 ml/kg) orally as control. Group II was taken as the standard group and received Furosemide (10 mg/kg) orally. Group III and IV were the test groups and received test drug in doses of 200 mg/kg and 400 mg/kg orally. Drugs were given to the animals after overnight fasting and subsequently animals were kept fasting during the experiment. Following drug administration, animals were immediately kept in specially designed metabolic cages for 24 hours. At the end of 24 hours collected urine was analysed for various electrolytes and its volume. Ethanolic extract of *Artocarpus heterophyllus* showed diuretic activity at the doses of 200 mg/kg and 400 mg/kg ( $p < 0.001$ ). The diuretic activity was more significant at the dose of 400 mg/kg. Ethanolic extract of *Artocarpus heterophyllus* has also demonstrated potassium sparing ability in the study.

**Keywords:** *Artocarpus heterophyllus*, Diuretic, Furosemide.

#### INTRODUCTION

Worldwide, the most common cause for morbidity is Cardiovascular Disease (CVD) accounting for 30% of deaths. This includes 40% in high income countries and 28% in middle and low income countries. Among the cardiovascular diseases, Hypertension solely accounts for 6% of deaths worldwide. Low dose diuretics are one of the commonly employed treatment approaches for treatment of hypertension<sup>1</sup>.

Diuretics are defined as drugs that will increase the excretion of Sodium along with water and decrease reabsorption of an anion, usually Chloride<sup>2</sup>. Various uses of diuretics include; treatment of edema caused due to cardiac, hepatic or renal origins, acute pulmonary edema, cerebral edema etc. One of the subclasses of diuretics, the high ceiling or loop diuretics are reserve drugs for patients with Congestive heart failure, reduced Glomerular filtration rate or sodium retention. Furosemide, is a high efficacy diuretic agent, effective even in patients with poor renal parameters or those who are not responding to other diuretics; but having significant adverse effects<sup>3</sup>. Adverse effects associated with Furosemide include electrolyte disturbances (Hypokalemia, Hyponatraemia etc.), hyperglycemia and hypersensitivity reactions<sup>4</sup>. These concerns have redirected our scientific research to a new path for exploring natural sources for even more favourable diuretics<sup>5</sup>. (Figure 1)



**Figure 1:** *Artocarpus heterophyllus*

*Artocarpus heterophyllus* (Jack fruit) is an evergreen tree seen in the tropical parts and throughout India<sup>6</sup>. It belongs to family Moraceae or mulberry family or the fig family. *Artocarpus heterophyllus* is known for its various medicinal properties like anti-fungal, anti-bacterial, ant-helminthic and anti-oxidant activity. It is also said to possess anti-diabetic and anti-inflammatory properties. The seeds are constipating in nature<sup>6-15</sup>. Ayurvedic literature has mentioned the use of seeds of *Artocarpus heterophyllus* as a diuretic agent<sup>16</sup>. Phytochemical analysis has shown that *Artocarpus heterophyllus* contains various phenols and flavonoid like morin, artocarpin, ursolic acid, betulinic acid which may be responsible for the diuretic activity<sup>16</sup>. The diuretics currently available have the potential to cause considerable adverse effects, which can in some cases be even life threatening. In the present study, we have attempted to explore the diuretic property of acute administration of Ethanolic Extract of seeds of *Artocarpus heterophyllus* (EEAH) on Albino Wistar rats.

**MATERIALS AND METHODS**

**Site of the study**

The study was conducted at A.J. Institute of Medical Sciences and Research Centre, Mangalore, India. The experimental protocol was approved by the Institutional Animal Ethics Committee (Approval No. IAEC/01/2015/CPCSEA). All experimental procedures were conducted in accordance with CPCSEA and OECD guidelines.

**Animals**

Twenty-four adult albino Wistar rats of both sex weighing 150-250 grams acquired from Central animal house of A. J. Institute of Medical Sciences and Research Centre were used. Animals were housed in clean polypropylene cages, maintained at standard laboratory conditions with light: dark cycle of 12: 12 hours and provided with food and water ad libitum. One-week period was allowed for the animals to acclimatize to these conditions and thereafter experiments were performed during the light phase of the cycle.

**Control and standard drugs used**

Normal saline was used as a control (vehicle) in the study. Furosemide (10mg/kg) was used as standard diuretic agent (Sanofi pharmaceuticals). All the drugs were procured from A.J. hospital Pharmacy.

**Test drug and preparation of plant extract**

EEAH was used as the test drug at the doses of 200 mg/kg and 400 mg/kg<sup>17</sup>.

Seeds of *Artocarpus heterophyllus* were collected from various parts of Mangalore and authentication was done. Seeds were shade dried and powdered. Dry powder of *Artocarpus heterophyllus* seeds was then kept for cold maceration with 95% ethanol. From the dry powder obtained 100gram powder was mixed with 300ml of ethanol and kept for 7 days with intermittent agitation. At the end of 7<sup>th</sup> day the extract was filtered, cooled and kept at room temperature<sup>18</sup>.

The ethanolic extract hence obtained was freshly dissolved in normal saline prior to administration and doses were given to the animals orally.

**Experimental design**

Rats of either sex were divided into four groups of six animals each using simple randomization method as shown in Table 1.

Group I and II were kept as control and standard groups whereas Groups III and IV were the test groups.

All the drugs were dissolved in normal saline and administered orally once to rats of each group prior to the experiment.

**Evaluation of diuretic activity**

Lipschitz et.al<sup>19</sup> method was used for evaluating the diuretic activity of the extract. Following overnight fasting animals were given normal saline at a dose of 2.5 ml/kg orally before commencing the experiment. This was to ensure uniform salt and water load. Following this drugs were administered to the animals orally. Immediately after drug administration, animals were placed in individual metabolic cages which have a mesh to separate the urine from faeces, for 24 hours. Animals were kept fasting during the experiment. End of 24 hours, urine was collected and was measured for its volume and electrolyte (Sodium, Potassium and Chloride) concentrations<sup>20</sup>. (Figure 2)



**Figure 2: Metabolic cage**

$$\text{Diuretic activity} = \frac{\text{Urinary excretion of test drug group}}{\text{Urinary excretion of control group}}$$

**Statistical Analysis**

Data were expressed as mean ± SEM. The results were analyzed by using one-way ANOVA followed by Tukey Kramer test. P value of <0.05 was considered as statistically significant. Graphpad Instat V3.10 software was used for the statistical analysis.

**Table 1: Groups and drugs administered and dose of the drugs in each group**

Groups (n=6)	Drug Administered	Dose
Group I	Normal saline (Control)	10 ml/kg, orally
Group II	Furosemide (Standard)	10 mg/kg, orally
Group III	EEAH (Test drug)	200 mg/kg, orally
Group IV	EEAH (Test drug)	400 mg/kg, orally

**Table 2: Effect of acute administration of EEAH on urinary volume and urinary electrolyte excretion**

Groups (n=6)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Volume (ml)
Group I	107.73±2.75	17.14±0.37	38.36±3.39	1.82±0.26
Group II	174.54±1.63***	13.45±0.49***	85.71±1.39***	5.83±0.46***
Group III	164.51±1.49***	8.11±0.34***	153.96±9.2***	4.03±0.29**
Group IV	165.89±1.91***	8.01±0.32***	164.89±10.82***	5.75±0.53***

n=6 The observations are mean ± SEM. \*\* p<0.01, \*\*\*p<0.001 as compared to control (One way ANOVA followed by Tukey Kramer comparison test) EEAH – Ethanolic Extract of seeds of *Artocarpus heterophyllus*.

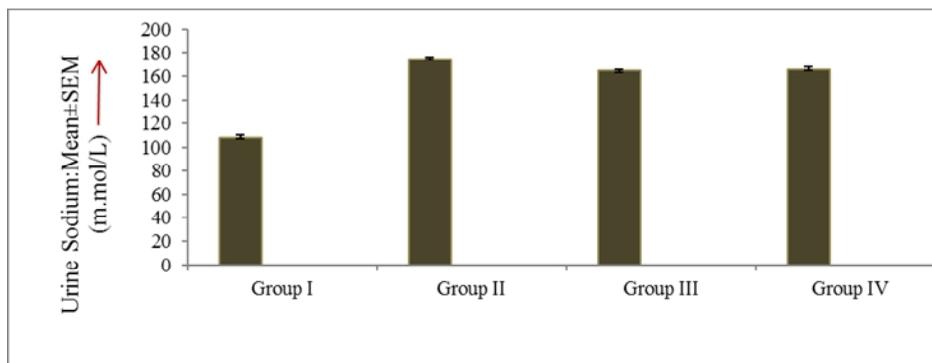


Figure 3: Effect of acute administration of EEAH on urine Sodium levels

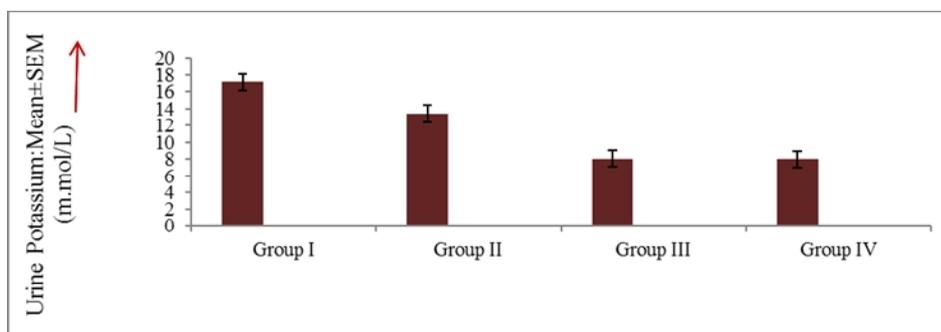


Figure 4: Effect of acute administration of EEAH on urine Potassium levels

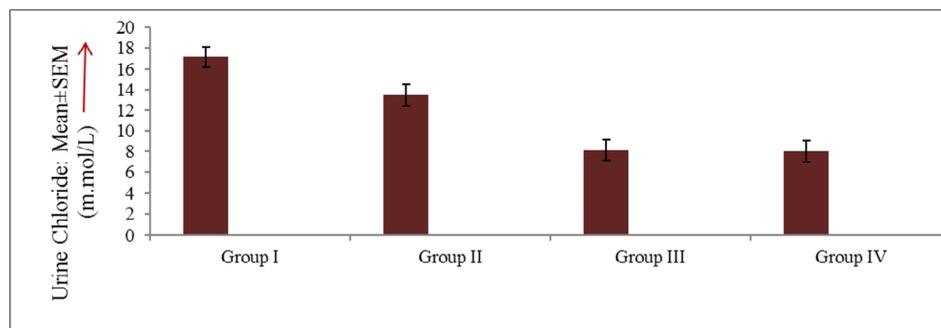


Figure 5: Effect of acute administration of EEAH on urine Chloride levels

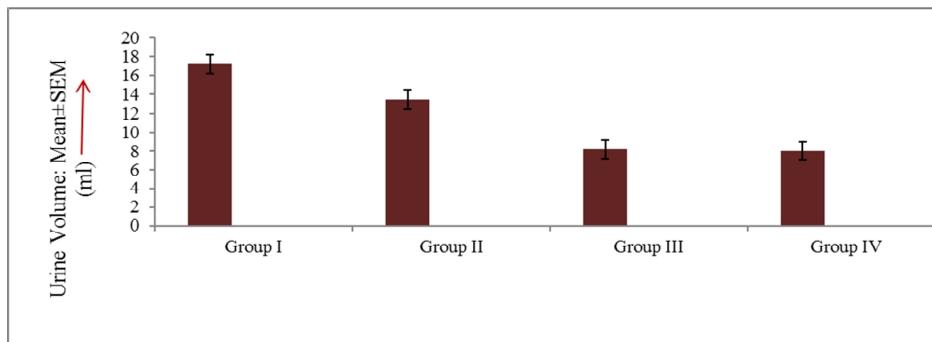


Figure 6: Effect of acute administration of EEAH on urine volume

## RESULTS

### Effect on urine volume and urinary electrolyte excretion

As shown in Table 2 and the Figures 3- 6; EEAH showed significant diuretic activity at the doses of 200 mg/kg and 400 mg/kg but the diuretic activity is more at 400 mg/kg dose. (Table 2), (Figures 3-6).

## DISCUSSION

Plant sources have always displayed great potential for providing newer medicines to humanity.<sup>21</sup> *Artocarpus heterophyllus* seeds are being used as one of the diuretic agents by the practitioners of Ayurvedic system of medicine<sup>7</sup>. But this data is not scientifically validated.

In this study the test group animals showed statistically significant diuretic activity compared to the control group and this value was comparable with that of the standard group at doses of 200 mg/kg and 400 mg/kg. The increase in the urine volume along with the alterations in the urinary excretion of electrolytes including Sodium, Potassium and Chloride proves the potential for diuretic activity of *Artocarpus heterophyllus* seeds. Furosemide being a loop diuretic also possesses the disadvantage of causing hypokalemia which necessitates the use of a potassium sparing diuretic or a potassium supplement along with Furosemide, especially in case of long term treatment with the same. In our study it was shown that *Artocarpus heterophyllus* seeds not only cause an increase in the urinary excretion of electrolytes like Sodium and Chloride, it also causes a decrease in the excretion of urinary Potassium.

Furosemide can cause hypersensitivity in patients allergic to Sulfhydryl(-SH) groups<sup>3</sup>. A detailed analysis to outline the structure of active ingredients in *Artocarpus heterophyllus* should be done to evaluate for a more favourable side effect profile. Phytochemical analysis has shown that *Artocarpus heterophyllus* contains a wide variety of phenols and flavonoids which have been shown to contribute to diuretic potential<sup>5</sup>. Further studies are required to explore the diuretic potential of seeds of *Artocarpus heterophyllus* along with the underlying mechanism.

## CONCLUSION

This study showed acute administration of seeds of *Artocarpus heterophyllus* has significant diuretic activity when tested on Wistar albino rats using Lipschitz method. Ethanolic extract of *Artocarpus heterophyllus* showed diuretic activity with potassium sparing activity at both 200mg/kg and 400mg/kg doses. But the diuretic activity at 400mg/kg is more compared to diuretic activity at the dose of 200mg/kg. The present study has shown an increased excretion of urine along with electrolytes like Sodium and Chloride and also a Potassium sparing action. Following a detailed analysis if the structure of active ingredient in *Artocarpus heterophyllus* shows no Sulfhydryl group then this along with the diuretic activity can be a promising drug in the future.

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