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Evaluation of the Diuretic Activity of *Alocasia macrorrhizos* in Rats.

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ABSTRACT

Alocasia macrorrhizos (AM) belongs to the family Araceae, different parts of this plant have shown hepatoprotective, anti-oxidant and anti-inflammatory action. Though it is traditionally used as a diuretic it needs substantial experimental evidence to support this. Hence we aimed to evaluate the diuretic activity of hydroalcoholic extract of leaves of *AM* in wistar rats. Acute diuretic activity of hydroalcoholic (50%) extract of leaves of *AM* (250 mg/kg and 500 mg/kg body weight orally) was studied in saline primed wistar albino rats (n=6). Furosemide (10 mg/kg) orally was used as the standard. Total 24 hours urine volume was measured using metabolic cages. The concentration of Na^+, K^+ in the urine at the end of 24 hours was estimated. Data was analyzed by One-way ANOVA followed by Dunnett test. Hydroalcoholic extract of leaves of *AM* showed a significant ($P < 0.05$) dose dependent increase in urine volume (8.1 ± 0.97ml/100g/24hr and 9.7±0.75 ml/100gm/24hr). At 500 mg/kg AM increased the excretion of sodium but decreased the excretion of potassium significantly compared to control. This preclinical study showed a potential diuretic activity but further studies regarding the mechanism of action is required to validate this finding.

Keywords: *Alocasia macrorrhizos,* diuretic, furosemide, potassium sparing effect,

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INTRODUCTION

Diuretics are agents which act on kidney and promote formation of urine and also help to eliminate of excess fluid and electrolyte from circulation [1]. Diuretic agents are used clinically in conditions like congestive heart failure, nephrotic syndrome, cirrhosis, renal failure, hypertension, and toxaemia of pregnancy [2]. Diuretics like furosemide and thiazides have been shown to have side effects, finding newer and safer therapeutic agents especially from indigenous plant sources would benefit the existing treatment modalities.

Alocasia macrorrhizos (AM) is an indigenous herb belonging to the family Aracea, Synonym: *Alocasia indica (Roxb.) Schott* and *Alocasia macrorrhiza* [3]**.** Different parts of this plant are traditionally used in inflammation. The extract of leaves of the plant is used in treatment for its digestive, laxative, diuretic, astringent effect [4]. It has been proved to have hepatoprotective properties [5], anti-inflammatory, antinociceptive and anti-oxidant properties [6]. Though this plant has been traditionally used as a diuretic, there is no experimental or clinical evidence to support it. Hence we aimed to experimentally confirm the diuretic activity of AM in wistar rats. Thus we planned this study with the objective of evaluating the diuretic activity of hydroalcoholic extract of leaves of AM.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of AM were collected from different places in Mangalore. It was identified and authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poornaprajna College, Udupi and a voucher specimen was deposited in the Herbarium of the institution.

Preparation of extract

Fresh leaves of AM were shade dried after which it was ground into coarse powder. Five hundred grams of this powder was extracted with ethanol (99%) and distilled water in 1:1 proportion at room temperature by cold maceration method [7]. The filtrate thus obtained was concentrated by heating at 45^0C. The extract was successively dried by using a rotary evaporator, with yield of the extract being 6.88% and this was preserved at $<0^0$C for subsequent use.

Experimental animals

Adult wistar rats of either sex, inbred in the institutional animal house were used for the study after acclimatization to standard laboratory conditions. Rats were housed in clean polypropylene cages, three rats in each cage, in a controlled environment (22-24^0C) with a 12 hour light and dark cycle with standard chow and water *ad libitum.* The study was conducted as per CPSCEA guidelines and prior approval was obtained from the Institutional Animal Ethics Committee.

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Study procedure

The method of Lipschitz *et al.*, [8,9] was employed for the assessment of diuretic activity. Twenty four rats deprived of food and water for 18 hours prior to the experiment, were divided into 4 groups (n=6) randomly. Each animal was placed in an individual metabolic cage 24 hours prior to the commencement of the study for adaptation. Before treatment, all animals received normal saline (0.9% NaCl) orally at a dose of 2.5ml/100g body weight to impose a uniform water and salt load [10] After this, the animals were treated orally with freshly prepared drugs as follows: Group I, (Control) received normal saline; Group II, (standard control) received furosemide (Sanofi Aventis Co.) 10 mg/kg, orally; Group III (AM 250) received hydroalcoholic extract of AM leaves 250mg/kg/day and Group IV (AM 500) received hydroalcoholic extract of AM leaves 500mg/kg/day. After dosing, each animal was placed in the metabolic cage, specially designed to separate urine and faeces, kept at 20°C±0.5°C. The total volume of urine collected was measured at the end of 24 hours. During this period, no food and water was made available to the animals. Concentration of Na^+, K^+ in the urine was determined by Ion Sensitive Electrode; Roche Hitachi 917 automatic analyzer. The diuretic action of test drug was calculated by using the following formula:

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

Statistical Analysis

The results were expressed as mean ± SD. The data was analyzed by One-way ANOVA followed by Dunnett test. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was carried out using the software package SPSS (Version 17.0).

RESULTS

The standard drug furosemide increased the urine volume at 24 hour interval to 9.83±0.75 ml which was significantly higher than that of control group (4.07±0.25; P<0.001). Hydroalcoholic extract of leaves of AM at the dose of 250mg/kg/day and 500mg/kg/day also showed a significant increase (P<0.001) in 24 hour urine output, 8.12±o.97 ml & 9.7±0.75 ml respectively when compared to control group (table 1).

The volume of urine at 24 hour interval for AM 500mg/kg treated group was comparable to that of furosemide 10 mg/kg (table 1). There was a dose dependent increase in urine output and diuretic index of 2 & 2.38 for AM 250mg/kg and 500mg/kg treated groups respectively.

Urinary electrolyte excretion

Furosemide treated group and AM 500mg/kg treated group showed significant increase in the urine sodium concentration at 24 hour interval (179±9.66, P<0.001 & 164.17±7.77, P<0.001 respectively) when compared to control group. Urine potassium level at 24 hour interval for furosemide (40.53±1.61) and AM 250mg/kg treated group were

comparable to control. Urine potassium level at 24 hour interval significantly decreased for AM 500mg/kg treated group (21.92±2.29, P<0.001) when compared to control. The urine sodium and potassium concentrations at 24 hour interval of AM 250mg/kg treated group were comparable to that of control group (Table 2).

Table 1: Effect of oral administration of hydroalcoholic extract of leaves of *Alocasia macrorrhizos* (AM) and furosemide on urine volume at 24 hours

Group (n=6)	Urine volume (ml/24hr)	Diuretic index (24 hr interval)[§]
Control	4.07±0.25	1
Furosemide 10 mg/kg	9.83±0.75*	2.42
AM 250 mg/kg	8.12±0.97*	2
AM 500mg/kg	9.7±0.75*	2.38

Values expressed in mean ± SD; *P <0.001 compared to control group (One-way ANOVA followed by Dunnett test)

[§]Diuretic index = volume of test group/volume of control group

Table 2: Effect of oral administration of hydroalcoholic extract of leaves of *Alocasia macrorrhizos* (AM) and furosemide on urine sodium and potassium

Group (n=6)	Na^+ mmol/L (Mean±SD)	K^+ mmol/L (Mean±SD)
Control	138.32±4.12	40.18±2.75
Furosemide 10 mg/kg	179±9.66**	40.53±1.16
AM 250 mg/kg	142.6±4.48	43.26±7.62
AM 500mg/kg	164.17±7.77**	21.92±2.29**

Values expressed in mean±SD; **P <0.001 compared to control group (One-way ANOVA followed by Dunnett test)

DISCUSSION

It was observed that AM at 250mg/kg showed diuretic effect without altering the excretion of urinary electrolytes. AM at 500mg/kg showed diuretic and natriuretic effect comparable to furosemide but significant decrease in urine potassium levels compared not only to furosemide but also to control. A previous study conducted on ethanolic extract of AM [11] showed diuretic activity at 400 mg/kg and also increased excretion of electrolytes. We studied the hydroalcoholic extract (50%) as some of the active constituents which may be water soluble would also be available in such an extract. And using a hydroalcoholic extract of AM in this study was justified by our results which showed diuretic and potassium sparing action at 500mg/kg, unlike the previous study. In accordance with a previous study furosemide at 10 mg/kg showed diuretic and natriuretic action but no kaliuresis [12]. Furosemide increases urine output and urinary excretion of sodium and potassium by inhibiting Na+ K+2Cl- co-transporter in the thick ascending loop of Henle [13] lowering plasma sodium and potassium levels. Hypokalemia on long term use of diuretics is problematic. In this scenario AM at 500mg/kg could be an ideal diuretic due to its natriuretic and potassium sparing action. The exact mechanism of action is difficult to comment on at this point of time. Further studies to elucidate the mechanism of action are necessary. AM contains several compounds like flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acid, succinic acid, β-lectines [14], but with the

present study it is difficult to comment on which of the compounds are responsible for the diuretic action.

CONCLUSION

This preclinical study in rats has proved diuretic action of *Alocacia macrorrhizos* at 500mg/kg dose with potassium sparing effect.

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