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Evaluation of diuretic activity of *Amaranthus spinosus* Linn. aqueous extract in Wistar rats

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ABSTRACT

Ethnopharmacological relevance: Traditional Siddha medicine literature claims that the *Amaranthus spinosus* Linn. (family: Amaranthaceae) whole plant possesses diuretic property.
Aim of the study: To evaluate the diuretic potential of *Amaranthus spinosus* aqueous extract (ASAE) in rats.
Material and methods: Different concentrations of ASAE (200, 500, 1000, 1500 mg/kg), thiazide (10 mg/kg) and vehicle were orally administered to rats ($n = 6$ animals per group) and their urine output was collected after 24 h. Volume, pH, Na^+, K^+ and Cl^- concentrations of urine were estimated.
Results: ASAE produced increase in Na^+, K^+, Cl^- excretion, caused alkalinization of urine, showed strong saluretic activity and carbonic anhydrase inhibition activity. These effects were observed predominantly at 500 mg/kg dose and there was no dose–response relationship.
Conclusion: Our study strongly suggests that the *Amaranthus spinosus* is acting as a thiazide like diuretic with carbonic anhydrase inhibitory activity which restates the claim as diuretic herb in Siddha medicine.

1. Introduction

Amaranthus spinosus Linn. (Amaranthaceae) is known as mullu keerai in Siddha medicine, which is a traditional system of medicine of South India. This herb is commonly known as prickle amaranthus. It is a branched erect herb, armed with axillary spines and ovate-elliptic leaves up to 12 cm × 6 cm. Unisexual flowers are seen in axillary clusters and in terminal of the plant (Gopalakrishna, 2003).

Literature in Siddha medicine claims that the decoction of *Amaranthus spinosus* has potent diuretic activity and reduces edema secondary to cardiovascular diseases and kidney diseases (Mudhaliyar, 1998; Varier, 2005). Its antimalarial (Hilou et al., 2006), anthelmintic (Kumar et al., 2010), anti-diabetic and anti-hyperlipidemic (Sangameswaran and Jayakar, 2005) activities have been confirmed by various studies. It also stimulates proliferation of B lymphocytes in vitro (Lin et al., 2005). It inhibits the spontaneous and dexamethasone induced apoptosis in murine primary splenocytes (Lin et al., 2008). It has also shown anti-inflammatory and analgesic activity (Hussain et al., 2009). Alcoholic extract of this plant showed a dose-dependent antidiarrheal and antiulcer activity in different animal model (Hussain et al., 2009).

Since a systematic study of its diuretic activity has not been undertaken, this study was done to evaluate the diuretic activity of *Amaranthus spinosus* aqueous extract (ASAE) in rats.

2. Material and methods

2.1. Plant material

The whole plant with root was collected from Manipal, India and authenticated by Department of Botany, MGM College of Science, Udupi, India. A voucher specimen (As/02, 2009) was deposited at the Department of Pharmacology, Kasturba Medical College, Manipal, India.

2.2. Amaranthus spinosus aqueous extract (ASAE)

Whole plant was collected, freshly washed thoroughly and air-dried in an oven at 40 °C for 5 days. The dried plant (500 g) was cut into small pieces, partially crushed and soaked in distilled water overnight. Then it was subjected to boiling for 6 h. The resultant

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extract was then drained and concentrated on water bath and desiccators sequentially to yield 9.2% of concentrated extract (46 g).

The chemical test of the product was done for testing the presence of saponins, anthraquinones, alkaloids, tannins and flavonoids (Yadav et al., 2010). The LD_{50} of ASAE was found to be above 2000 mg/kg orally (Kumar et al., 2008). To evaluate the dose related diuretic activity, we had chosen different doses of ASAE viz. 200 mg, 500 mg, 1000 mg and 1500 mg per body weight.

For the pharmacological study, different concentrations of extract were given orally to laboratory rats as a suspension in 8% gum acacia aqueous solution in a final volume of 5 ml/kg body weight.

2.3. Experimental animals

The study was approved by the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal (approval no: IAEC/KMC/50/2009-2010). Healthy adult male albino rats of Wistar strain weighing between 150 and 200 g were used. Animals were housed in standard environment conditions (temperature 28–30 °C, photoperiod; approximately 12 h natural light per day; relative humidity: 50–55%).

2.4. Drugs

Hydrochlorothiazide (HCTZ) (Sigma Chemicals Co.) was used as a reference diuretic drug.

2.5. Evaluation of diuretic activity

Male albino rats were divided into six groups, of six animals each, in laboratory cage. They were fed with standard laboratory diet ad libitum and allowed free access to drinking water. The animals were fasted overnight, with free access to tap water only. Four groups of animals were administered 5 ml/kg body weight of the fractions of ASAE orally and one group of rats received 5 ml/kg body weight of HCTZ 10 mg/kg orally. Control group rats also received the same amount (5 ml/kg body weight) of double distilled deionized water orally. Immediately after administration, the rats were placed individually in the metabolic cages. Urine was collected in a graduated cylinder.

At the end of 24 h, urine volume was recorded and urine output was calculated in relation to body weight and expressed as ml/100 g body weight. pH of urine was noted by using litmus paper (Glassco, India). Electrolyte (Na^+, K^+, Cl^-) concentrations were estimated and expressed as mmol/L. Each animal was given two trials at two weeks interval and the average values were taken.

2.6. Analytical procedure

Estimation of electrolytes was performed according to the procedure provided along with electrolyte estimation-standard-reagents kit (Crest Biosystems, India). The Na^+, K^+, Cl^- concentrations were measured using GENESYS 10 UV spectrophotometry (Thermoelectron Corporation, USA).

Sum of Na^+ and K^+ was calculated as parameter for saluretic activity. The ratio of Na^+/K^+ was calculated for natriuretic activity. To estimate carbonic anhydrase inhibition activity, the ratio of $Cl^-/(Na^+ + K^+)$ was computed (Vogel, 2002).

2.7. Statistical analysis

Statistical work was done by using SPSS software version 11.5. Values are expressed as mean ± SEM. The statistical evaluation was carried out by analysis of variance (ANOVA) with post hoc test Tukey alpha (0.05). Significance was set at $P \leq 0.05$.

3. Results

3.1. Chemical tests

Chemical tests were done in our lab which showed the presence of saponin and anthraquinones in the ASAE. Alkaloids, tannin, and flavonoids were absent in our test.

3.2. Urine output volume and pH

The ASAE did not produce dose dependent urine output, but maximum efficacy (34%) was obtained at 500 mg/kg dose, which was close to the reference drug HCTZ (38%). Diuretic index of ASAE was similar to HCTZ at doses of 500 mg and 1000 mg/kg. At dose of 500 mg/kg, ASAE showed high urinary pH which was statistically significant when compared to control group ($P = 0.016$) and thiazide group ($P = 0.047$) (Figs. 1 and 2).

3.3. Electrolyte excretion

Figs. 3 and 4 show the electrolyte excretion after oral administration of ASAE, HCTZ and control groups. ASAE at 500 mg/kg dose excretes Na^+ significantly ($P < 0.0001$) compared to control and HCTZ groups. ASAE at doses 500 mg/kg and 1500 mg/kg showed significant increase in Cl^- excretion compared to control group. Potassium excretion values showed that 500 mg/kg and 1500 mg/kg ASAE produced high K^+ excretion when compared to control group as well as HCTZ group (Table 1).

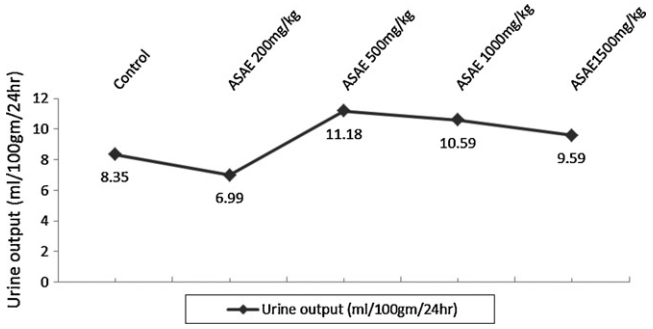

Fig. 1. Dose related effect of *Amaranthus spinosus* aqueous extract (ASAE) on urine output in 24 h of urine collection. Drugs were given orally ($n = 6$). Histograms represent mean urine output (ml/100 g/24 h).

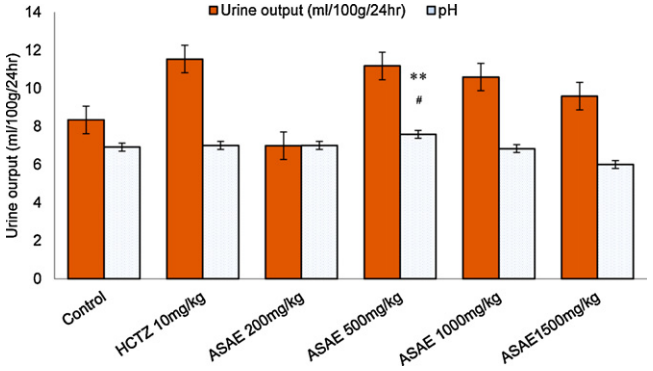

Fig. 2. Effect of *Amaranthus spinosus* aqueous extract (ASAE) and hydrochlorothiazide (HCTZ) on urine output and pH in 24 h of urine collection. Drugs were given orally ($n = 6$). Histograms represent mean ± S.E.M. $^{**}P = 0.016$ vs. control; $^{\#}P = 0.047$ vs. HCTZ.