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Research Article

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Evaluation of Diuretic Activity of Ethanolic Extract of *Cassia auriculata* Seeds

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ABSTRACT

Objective: Evaluation of diuretic activity of ethanolic extract of cassia auriculata seeds. Methods: ethanolic extraction of the plant by maceration and the parameters tested during the experimental on each mouse were: total urine volume and the concentration of sodium, potassium, and chloride ions in urine. Results: it showed diuretic activity by inhibiting tubular reabsorption of water and anions. Conclusion: From the results it is evident that, the crude ethanolic extract of Cassia auriculata seeds is safe to use as a diuretic.

Keywords: *Cassia auriculata*; Diuretic activity; Urine output

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INTRODUCTION

Diuretics are the drugs that enhances the urine formation by kidney by decreasing Na^+/H_2O reabsorption. These agents are potent in treatment of disorders that exhibit edema such as Congestive heart failure, nephritis, toxemia of pregnancy, hypertension and premenstrual tension [1]. However majority of allopathic medicines used today are associated with adverse drug reaction. In order to overcome ADR the world today is carrying out many research works on herbal drugs. *Cassia auriculata* is one of the herbs of great medicinal value containing many phytochemicals of significant use. The flowers are used in throat irritation [2], both types of diabetes mellitus, pharyngopath [3], nocturnal emissions and urinary discharges. The roots are astringent, cooling, and are useful in asthma, tumors, skin diseases, leprosy, tumors and urethrorrhea. Seeds are astringent, sour, constipating, depurative, aphrodisiac, antihelmintic, stomachic, useful in diabetes, dysentery, diarrhoea, leprosy, abdominal disorders, worm infestations, chronic purulent conjunctivitis [4] and anxiolytic activity [5]. In addition, *Cassia auriculata* has been widely used in ayurvedic medicine. The present study was carried out in an attempt to evaluate the diuretic effects of *Cassia auriculata*.

MATERIALS AND METHODS

Animals

In this diuretic activity, Albino Mice (20 gm) were used. They were obtained from in house facility (Bhaskar medical college, Hyderabad) and the study was performed under standard conditions in accordance with CPCSEA guidelines.

Preparation of Extract

Cassia auriculata, plant seeds were collected from surrounding areas of Ananthagiri, Telangana, India and authenticated by Department of Botany, Telangana University. The extract was prepared by using ethanol, by maceration method for 72 hrs at room temperature and was concentrated by simple evaporation at room temperature.

A suspension of CSE in water was prepared for oral administration. The study of phytochemical analysis was performed [6].

Acute Toxicity Study
The acute oral toxicity of the *Cassia auriculata* seeds extract (CSE) was determined in fasted albino mice by fixed dose method according to OECD guidelines No. 423**.**

Diuretic Activity
In this method, albino mice of either sex were used. The mice were divided five groups (n=6) and were fasted for 24 hours prior to the experiment.
Group 1: Control- Normal saline .p.o
Group 2: Furosemide - Standard drug 2 mg/kg .i.o
Group 3: CSE 250- Test 250 mg/kg. p.o.
Group 4: CSE 500- Test 500 mg/kg. p.o.
Group 5: CSE 1000- Test 1000 mg/kg. p.o
After administration the animals were placed in a metabolic cage (2 per cage), specially designed to separate urine and collected was measured at the end of 5hours. During this period, no food and water was made available to animals. The volumes of urine, electrolytes like Na+, K+, Cl- were estimated in urine for assessment of diuretic activity [7].

Statistical Analysis
The experimental data were expressed as mean ± SEM (n=6). Statistical analysis was performed with one-way-ANOVA followed by Dunnett's t-test.

RESULTS

The preliminary photochemical test revealed the presence of flavonoids, amino acids, glycosides and alkaloids in *Cassia auriculata* seed extract.

Acute Toxicity Study
The albino mice were fasted overnight; the *Cassia auriculata* seed extract was administered by gastric intubation to the animals orally at 50 mg/kg body weight up to the dose 5000 mg/kg. Acute oral toxicity studies were performed as per OECD-423 guidelines. No considerable sign of toxicity observed in treated animals. No mortality observed in all test animals when they were subjected to acute toxicity studies upon oral administration of 5000 mg/kg.

Table 1: Diuretic Effect of ethanolic extracts of *Cassia auriculata* in mice

Treatment	Urine volume (ml/100g/5h)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
Control	4.32 ± 0.58	101.20 ± 1.18	91.18 ± 0.63	57.32 ± 5.18
Furosemide (20mg/kg)	$8.43 \pm 0.48^{***}$	$124.62 \pm 0.16^{**}$	$62.04 \pm 2.14^{***}$	$78.24 \pm 1.64^{*}$
250mg/kg of CSE	5.43 ± 0.28^{ns}	111.46 ± 3.47^{ns}	89.21 ± 2.80^{ns}	55.27 ± 3.18
500mg/kg of CSE	$6.70 \pm 0.22^{*}$	$117.24 \pm 3.16^{**}$	$78.61 \pm 1.28^{***}$	60.71 ± 5.20
1000mg/kg of CSE	$7.58 \pm 0.62^{***}$	$121.64 \pm 3.47^{***}$	$72.47 \pm 1.30^{***}$	62.47 ± 5.65

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by Dunnett's test)

As mentioned in Table 1 the Control, CSE (250 mg/kg, 500 mg/kg and 1000 mg/kg) and standard drug of urine volume were 4.32 ± 0.58, 5.43 ± 0.28, 6.70 ± 0.22 and 7.58 ± 0.62, 8.34 ± 0.48 respectively. Estimation of Na^+ (m mol/L) of Control, CSE (250 mg/kg, 500 mg/kg and 1000 mg/kg) and standard drug were 101.20 ± 1.18, 111.46 ± 3.47, 117.24 ± 3.16 and 121.64 ± 3.47, 124.62 ± 0.16 respectively. Estimation of K^+ (mmol/L) of the Control, CSE (250 mg/kg, 500 mg/kg and 1000 mg/kg) and standard drug of urine volume were 91.18 ± 0.63, 89.21± 2.80, 78.61 ± 1.28 and 72.47 ± 1.30, 62.04 ± 2.14, respectively. Estimation of Cl^- (mmol/L) of the Control, CSE (250 mg/kg, 500 mg/kg and 1000 mg/kg) and standard drug were 57.32 ± 5.18, 55.27 ± 3.18, 60.71 ± 5.20 and 62.47 ± 5.65, 78.24 ± 1.64 respectively. The extract was found to produce significant increase in excretion of all ions at the higher dose (1000 mg/kg p.o). The urinary output increased in dose dependent manner.

DISCUSSION

Diuretics are drugs which decrease Na^+/ H_2O reabsorption and thereby reduce abdominal accumulation of excess fluid in body. These help in decrease in cardiac work load by reducing blood volume and venous return to the heart. They have wide application in management of cardiovascular diseases such as congestive heart failure and hypertension, certain renal diseases, peripheral edema and pulmonary congestion. They are also useful in reducing syndromes of volume overload including paroxysmal nocturnal dysopnea and orthopnea. Apart from its medicinal value, *Cassia auriculata* has also served the community as a source of food for centuries. With this prior information of safety limit test was found to be the appropriate test for assessing the acute toxicity profile as per the OECD guidelines. When the urine output measurement has done with 30 min interval for 5 hours using ethanolic extract of *Cassia auriculata* seeds had found to show a strong diuretic activity, a significant urine output as compared to control was observed starting from the first 2 hours ($P<0.001$) at the screening dose of 500mg/kg specifically. Although it fails to reach a statistically significant level, it shown slightly higher cumulative urine output compared to standard only at the dose of 500 mg/kg. The electrolyte excretion potential of *Cassia auriculata* seeds on Na^+ and Cl^- was significant in comparison to control group. Although a magnified excretion of both the ions compared to control had observed they could not cause significant k^+ excretion which was found significant only at 1000 mg/kg ($p<0.01$) compared to control. When compared to normal saline, all the extracts increased urine elimination and Na^+, K^+ and Cl^- excretion. The extract acts synergistically to the mechanism of [N^+/H^+] antiporter, [$HCO3^+/H^+$], [$HCO3^+/H^+$] exchangers resulting in diuresis. The diuretic activity of CSE exerted may be by inhibiting tubular reabsorption of water and anions.

CONCLUSION

As evidenced by this outcome of this study, it is safe to infer that the crude ethanolic extract of *Cassia auriculata* seeds possess a diuretic activity. As emphasized, diuretic property of CSE could be due to phytochemicals such as alkaloids, flavonoids, saponins and glycosides. The precise sites and the molecular and cellular methods of CSE action remain to be discussed in further studies.

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