

EVALUATION OF THE DIURETIC AND URINARY ELECTROLYTE EFFECTS OF ETHANOLIC EXTRACT OF WHOLE PLANT OF *GARDENIA GUMMIFERA*

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ABSTRACT

Diuretics are the agents which increase the rate of urine flow and sodium excretion and are used to adjust the volume and/or composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis. These agents were widely explored in Indian ancient system of medicine. The numerous diuretic plants with their active phytoconstituents have been explored. The present study was carried out to investigate the diuretic activity of ethanolic extract of aerial parts of *Gardenia gummifera*. Lipschitz test in Wistar rats was used to evaluate diuretic effect of ethanolic extract of *Gardenia gummifera*

with furosemide as a standard and normal saline as control. The urine volume (in mL) measured at 5 h. The urine volume and urinary electrolyte excretion (Na⁺ and K⁺) were found to be significantly higher in rats treated with *Gardenia gummifera* as compared to normal rats. This present study indicates that Ethanolic extract of aerial parts of *Gardenia gummifera* has potential diuretic and naturetic property.

KEYWORDS: *Gardenia gummifera*, Lipschitz test, diuretic activity, urine volume.

INTRODUCTION

Gardenia gummifera also known as gummy Cape jasmine is a species of plant in the Rubiaceae family which is widely distributed in central and southern India. The leaf buds yield a transparent, bright yellow gum-resin Gum exhudate called 'Dikkamaly resin'. Preliminary phytochemical screening of petroleum ether, ethyl acetate, methanol, ethanol and aqueous extracts showed the presence of various phytocompounds like glycosides,

phytosterols, fats and oils, phenols, resins, tannins, flavonoids, tannins and terpenoids.^[1] The phytochemical studies on the plant showed the presence of resins like gardenin, nevadensin, wogonins, isocutellarein, apigenin and de-MeO-sudachitin.^[2] The Dikkamaly resin is acrid bitter thermogenic, antispasmodic, expectorant, appetizer, anodyne, vulnerary anthelmintic, cardioprotective, revulsive and depurative. In Ayurveda it is used for the treatment of neuropathy, hiccup, cough, bronchitis, anorexia, dyspepsia, flatulence, colic, constipation, foul ulcer, wounds, intestinal worms, cardiac debility, leprosy, skin diseases, intermittent fever, splenomegaly and obesity.^[3] The different parts of plant has been screened for pharmacological activities and reported to possess analgesic^[4], anti-inflammatory^[4], antipyretic^[4], Antihelmenthic^[4], Cytotoxic^[5], antioxidant^[6], Antihepatotoxic^[6], Antiulcer^[7], Cardioprotective^[8], Antibacterial^[9], antimicrobial^[10], antiradical^[10] and insecticidal activity.^[10] The present study has been carried out to explore the diuretic activity of ethanolic extract of aerial parts of *Gardenia gummifera*.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves and flowers of the young and matured plants *Gardenia gummifera* belonging to the family Rubiaceae were collected in bulk from local area of Warangal, Telangana, India in the month of December 2016. The identification and the authentication of this plant were done in the Department of Botany, Kakatiya University.

Preparation of plant extract

After collection, leaves and flowers were washed separately very carefully and clearly with water and dried under shade. The dried leaves and flowers were powdered in an electrical processor. 50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of ethanol. The ethanolic extract was then distilled, evaporated and dried in vacuum to get semisolid resinous extract. All the extracts were kept in desiccator and stored in a refrigerator for further pharmacological experiment.

Animals

Wistar rats of either sex, weighing about 180-200 grams were used in experiments. Animals were housed in polypropylene cages with not more than three animals per cage and maintained under standard condition (12 hours light / dark cycle; temperature $25 \pm 3^{\circ}\text{C}$; relative humidity $55 \pm 5\%$) and had free access to standard pellet feed (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for a

week before commencement of experiment. The experiments on animals were conducted in accordance with CPCSEA and our protocols were duly approved by the Institutional Ethical Committee.

Acute Toxicity Studies

The acute toxicity study to carry out the gross behavioral effects and safety effects of the ethanolic extract of *Gardenia gummifera* was carried on mice weighing about 20-25gm as per ICH Topic S7A guidelines and OECD 423 guidelines. Overnight fasted mice received the test extract at a dose of 5 mg/kg bodyweight orally and mortality was observed for first 24 hours, with special attention for the first 4 hours and daily then, for a total of 14 days. If no mortality was observed for any mice, then the procedure was repeated again with doses of 50, 300 and 2000 mg/kg orally. The extract was well tolerated by the mice without any explicit signs of toxicity.^[11]

EVALUATION OF DIURETIC ACTIVITY BY LIPSCHITZ METHOD^{[12][13]}

The Lipschitz test has been demonstrated to be a standard method and a very valuable tool for screening of potential diuretics. Wistar rats were randomly divided into three groups of six animals each. Group 1 was served as the normal control group and treated with Normal Saline alone; Groups 2 served as the positive control and treated with furosemide (10 mg/kg, p.o.); Group 3-was treated with ethanolic extract of *Gardenia gummifera* (500 mg/kg, p.o). Animals were fasted and deprived of water for 18h prior to the experiments. On the day of experiment before treatment, all animals received 0.15 ml/10 gram body weight of 0.9% NaCl by oral gavage to impose a uniform water and salt load. Before the administration of the extract/controls, the bladder of the rat was emptied by gentle compression of pelvic area and pulling of tails. Immediately after dosing, the rats were placed in the metabolic cages (Three rats are placed in one metabolic cage) specially designed to separate urine and feces. Animals were kept at room temperature of temperature $25 \pm 3^{\circ}\text{C}$ and the relative humidity $55 \pm 5\%$ throughout the experiment period. The urine was collected in measuring cylinder upto 5hrs after dosing. During this period, food or water was not provided to the animals. The total volume of urine collected was measured for both control and treated groups. Urine volume excreted per 100 g body weight is calculated for each group.

$$\text{Diuretic index} = \frac{\text{Mean urine volume of the test group}}{\text{Mean urine volume of the control group}}$$

$$\text{Lipschitz value} = \frac{\text{Mean urine volume of the test group}}{\text{Mean urine volume of the reference group (Furosemide Group)}}$$

Results are expressed as the “Lipschitz-value” and indices of <0.72, 0.72–1.00, 1.00–1.5, and >1.5, will be regarded as will be considered “nil”, “little”, “moderate” and “good”, diuretic activity.^[14]

Urine electrolytes (sodium, potassium concentrations were measured using flame photometry.

STATISTICAL ANALYSIS

Values were expressed as Mean \pm Standard Deviation. The Significance of differences among the group was assessed using one way analysis of variance (ANOVA). The test followed by Dunnett’s multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant.^[15]

RESULT

This study has been carried out to establish the diuretic properties of ethanolic extract of aerial parts of *Gardenia gummifera*. For the screening of diuretic effect Lipschitz method was used which is based on water and sodium excretion in test animals and compared to rats treated with standard diuretic. Ethanolic extract of *Gardenia gummifera* (2.53 \pm 1.07) as well as furosemide(3.41 \pm 1.39) significantly increased the urine volume when compared with control 1.78 \pm 0.65 (Table 1) and showed significant diuretic effect. The diuretic activity of a drug is considered nil if it is less than 0.72, little if it is between 0.72 and 1.00, moderate if it is within 1.00–1.50 and good if it is above 1.50. In this respect, *Gardenia gummifera* extract has little significant diuretic activity when compared to that of control.

Table 1. Effect of oral administration of *Gardenia gummifera* extracts on urine volume

S. No	Treatment	Urine volume (ml/100g/5h)	Diuretic index	Lipschitz value
1	Control (Saline)	1.78 \pm 0.65	-	-
2	Standard (Furosemide)	3.41 \pm 1.39**	1.91	-
3	<i>G. gummifera</i> Extract	2.53 \pm 1.07*	1.32	0.74

Values are mean \pm SD (n=6) One way ANOVA followed by Dunnet’s test. *P < 0.05 and **P < 0.01 when compared to control

Further, the present results suggest that ethanolic extract of aerial parts of *Gardenia gummifera* increases the urinary electrolyte excretion of sodium and potassium ions and exhibit potent natriuretic activity when compared to normal control. (Table 2).

Table 2. Effect of oral administration of *Gardenia gummifera* extracts on electrolytic excretion

S. No	Treatment	Urinary Na ⁺ (mmol/L)	Urinary K ⁺ (mmol/L)
1	Control (Saline)	109.73±3.24	53.74±1.61
2	Standard (Furosemide)	171.58±2.98**	97.06±2.83
3	<i>G. gummifera</i> Extract	124.49±3.86*	69.23±1.26

Values are mean ± SD (n=6) One way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

DISCUSSION

Diuretics are the agents the increased production of urine and are used to treat heart failure, liver cirrhosis, moderate hypertension, influenza, water poisoning and certain kidney diseases.^[16] The medicinal plants constitute an everlasting source of active substances; those of diuretic action stand out due to its employment in the treatment of important diseases. The present study was undertaken to evaluate the diuretic activity of ethanolic extract of aerial parts of *Gardenia gummifera*. This study revealed that *Gardenia gummifera* extract significantly increased the urinary output which denotes that the plant has significant diuretic effect. Increase in the urinary volume was also accompanied by an increase in the excretion of sodium and potassium ions thus; *Gardenia gummifera* extract has been shown to possess significant saluretic and natriuretic effects. The diuretic effect of the *Gardenia gummifera* extract may be by Inhibiting vasopressin secretion or Inhibiting the Na-K-2Cl symporter, Na⁺/K⁺ exchanger or Na⁺/Cl⁻ symporter there by displaying the diuretic property.^[17] Diuretics are tools of considerable therapeutic importance. In the light of the above mentioned study, we can report that the ethanolic extract of aerial parts of *Gardenia gummifera* is an effective diuretic and also resulted in increased sodium, potassium and chloride ions in urine; it can be used as tools of considerable therapeutic importance for the management of Hypertension and congestive heart failure.

CONCLUSION

The present observations provide evidence that ethanolic extract of aerial parts of *Gardenia gummifera* showed significant diuretic property as it increased urine and electrolyte excretion. However, further studies are suggested for explaining the mechanism of diuretic activity.

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