

EVALUATION OF DIURETIC ACTIVITY OF *Gmelina arborea* ROXB. FRUIT EXTRACTSBHABANI SHANKAR NAYAK^{1*}, SUBAS CHANDRA DINDA¹, P. ELLAIAH²

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ABSTRACT

Gmelina arborea roxb. is extensively used traditionally as anthelmintic, antimicrobial, antidiabetic, diuretic, hepatoprotective and antiepileptic agent. The present study is an attempt to explore and confirm the diuretic activity of different fruit extracts of the plant *G. arborea* using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. The diuretic activity of the extracts were evaluated by determining urine volume, urine pH and electrolyte (Na⁺) concentration in male Wistar rats at a single dose of 300 mg/Kg, b.w. Urea (1 g/Kg) was used as normal standard drug and normal saline water was 0.9 % w/v used as normal control. The extracts were found to be nontoxic. Urine volume was significantly increased by all the extracts in comparison to normal and standard control groups. The excretion of sodium was also increased by all the extracts. There was no significant change in the pH of urine after administration of the *G. arborea* extracts. The diuretic effect of the extracts was comparable to that of the reference standard (Urea). The n-butanol extract showed better diuretic effect in comparison to other extracts. Thus it could be concluded that the fruit extracts of *G. arborea* possess diuretic activity.

Keywords: *Gmelina arborea*, Lipschitz value, diuresis, *Verbenaceae*.

INTRODUCTION

Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxemia¹. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness². Although most of the diuretics proved to be very effective in promoting sodium excretion, all cause potassium loss and this prompted the search for potassium sparing diuretics^{1,2}. Hence a search for new diuretic agents that retain therapeutic efficacy and yet devoid of potassium loss is justified.

The fruits of the plant *Gmelina arborea roxb.* are oval in shape, ¾ inches in length and are yellow in color. The fruits are sweet in taste and some times astringent^{3,4}. The plant, *G. arborea* was reported to have several medicinal properties such as aphrodisiac, astringent, analgesic, antipyretic, antidiabetic, diuretic, anti-inflammatory and tonic characteristics⁵.

The literature survey reveals that fruits of *G. arborea* contain cardiac glycosides and steroids. The ethanol extract contains alkaloids, carbohydrates, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds and flavonoids. The ethyl acetate extract contains gums, mucilages, proteins and amino acids. The n-butanol extract contains alkaloids, anthraquinone glycosides, gums, mucilages, tannins, phenolic compounds, triterpenoids, saponins and flavonoids. The petroleum ether extract contains alkaloids, carbohydrates, anthraquinone glycosides, proteins, amino acids, triterpenoids and saponins⁶.

MATERIALS AND METHODS

Drugs and Chemicals

The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. Urea, n-butanol GR 80°C and petroleum ether AR 40-60°C were procured from Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals and reagents were procured from authorized dealer.

Collection of plant materials, identification and size reduction

The fruits of *G. arborea* were collected from local area of Koraput district (India) in the month of April and May 2008. The plant was identified and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter no. MJ/DBT (08)/1067, dated 05.09.2008). The fruits were shade dried under normal environmental conditions. The dried fruits were pulverized

to form coarse powder by using electrical grinder and stored in a closed air tight container for further use.

Preparation of solvent extracts

The coarse powder form of dried fruits was extracted by Soxhletion method by using ethanol, ethyl acetate, n-butanol and petroleum ether as solvents. In this extraction process, a total amount of 1500 gm coarse powdered fruits was extracted with 1200 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.

Acute toxicity studies

To study the toxic effect (if any) of *G. arborea* fruit extracts, Albino mice of either sex weighing 20-25 g were used. The animals were kept in the standard polypropylene cages at 25±2°C/ 60% relative humidity in normal day and night photo cycle (12: 12 h). The animals were acclimatized for a period of 14 days prior to performing the experiments. Prior to the study, the experimental protocols were approved by the Institutional Animal Ethics Committee of Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No 1339/ac/10/CPCSEA).

Acute oral toxicity study was performed as per OECD-423 guidelines^{7,8}. The animals were kept fasting overnight but allowed free access to water *ad libitum*. The fasted mice were divided into different groups of six animals each. Each solvent extract solution was administered orally at a dose of 10 mg/Kg b.w., using normal saline water as vehicle and mortality in each group was observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the same procedure was repeated in each group for each extract for further higher doses such as 100, 300, 600, 1000, 2000 and 3000 mg/Kg b.w. One tenth of this lethal dose was selected as the therapeutic dose for the evaluation of antiepileptic and diuretic activities.

Diuretic activity (Lipschitz test)

Healthy adult Wistar male rats of weighing 150-200 g procured from the animal house of Gayatri College of Pharmacy, Sambalpur, Odisha, were used for the study. The animals were maintained in well ventilated room temperature with natural 12 + 12 day - night cycle in the polypropylene cages. The animals were fed with balanced diet that is standard rodent pellet diet (Hindustan Lever Ltd.) and water

ad libitum. The animals were housed for 1 week prior to the experiment to acclimatize to the laboratory conditions. Approval for the research work and ethical clearance was obtained from the Gayatri College of Pharmacy, Gayatri Vihar, Jamadarpali, Sambalpur, Odisha (Ethical Committee No 1339/ac/10/CPCSEA).

Wistar rats were divided into six groups (3 each). The animals of group (I) served as normal control (Vehicle) which received normal saline water (2 ml/kg b.w., orally) only. The animals of group (II) served as standard control which received Urea (1 g/kg b.w., orally). Groups (III) to (VI) received ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively at dose of 300 mg/kg b.w., orally. The method is based on water and sodium excretion in test animals as compared to rats treated with high dose of urea. The method of Lipschitz *et al.* was employed for the assessment of diuretic activity⁹⁻¹¹.

Male Wistar rats weighing 150 to 200 g were used. They were placed in metabolic cages provided with a wire mesh bottom and a funnel for collecting the urine. Stainless steel sieves were placed in the funnel to retain the feces, allowing only urine to flow down for collection and measurement. The food and water are withdrawn 15 h prior to the test. Three animals were placed in one metabolic cage. The rats of each group were treated with drugs as per the details mentioned above. Additionally 5 ml of normal saline solution per 100 g was administered orally to all rats. Urine excretion was recorded after 5 h. The sodium and potassium contents of the collected urine were estimated by Flame Photometer (Toshniwal group model TCM-35). The instrument was calibrated with standard solutions containing different concentrations of Na⁺ and K⁺. The conductivity was directly determined on fresh urine samples using a conductometer (Toshniwal group model TCM-15). pH was measured with a digital pH meter (MK-VI, Unique instruments & machineries, Calcutta) on fresh urine sample.

For the calculation and presentation of results, urine volume excreted per 100 g body weight was worked out. The results are expressed as "Lipschitz value" that is the ratio T/U, where T represents the response of the test compound and U that of urea treatment. The Lipschitz values were calculated for urine excretion. The indices of 1.0 and more indicate a positive diuretic effect of test compounds.

Statistical analysis

For determining the statistical significance, standard deviation, standard error mean and Dunnett's test 1% level significance was employed¹².

RESULTS AND DISCUSSION

Acute toxicity study

The acute toxicity studies revealed that there were no mortalities with any solvent extract at any dose in Swiss albino mice, which confirmed that *G. arborea* fruit extracts would be non-toxic in living body but where as the LD₅₀ of the extracts was found to be 300 mg/Kg body weight. One tenth of this lethal dose that is 300 mg/Kg b.w. was selected as the therapeutic dose for the evaluation of pharmacological activities.

Diuretic activity

All the four extracts were screened for their diuretic activities by administering orally at the dose of 300 mg/kg. The sodium and potassium concentrations, volume of urine and pH of urine were recorded. The ratio of the concentration of Na⁺/K⁺ and diuretic index at the end of 5 h were calculated to assess the diuretic activity. Table 1 shows the electrolyte (Na⁺ and K⁺) content (mmol/l/5h) of the urine of the animals. Table 2 shows the urinary volume (ml/100g/5h) and pH.

Table 1: Summary of parameters of diuretic activities of different extracts of *G. arborea* fruits.

| Groups | Dose | Na ⁺ (mmol/l) (X±SD) | K ⁺ (mmol/l) (X±SD) | Lipschitz value (Na ⁺) (T/U) | Na ⁺ / K ⁺ ratio | |
|---------------------|-----------|---------------------------------|--------------------------------|--|--|--------|
| I | 2 ml/kg | 109.6±1.25 | 67.82±1.17 | -- | 1.616 | |
| II | 1 g/kg | 121.34±1.08* | 77.61±1.05* | -- | 1.563 | |
| III | 300 mg/kg | 126.43±1.11* | 81.09±1.19* | 1.042 | 1.559 | |
| IV | 300 mg/kg | 124.22±1.21* | 79.23±1.21* | 1.023 | 1.568 | |
| V | 300 mg/kg | 133.62±1.09** | 84.32±1.02** | 1.102 | 1.585 | |
| VI | 300 mg/kg | 122.07±0.98* | 78.76±1.13* | 1.006 | 1.551 | |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 6005.4502 | 1 | 6005.45021 | 2.9321 | 0.00484 | 4.9646 |
| Within Groups | 464.38248 | 10 | 46.4382483 | | | |
| Total | 6469.8326 | 11 | | | | |

Data are presented in mean ± standard deviation, n = 3. Standard error of mean < 0.721. ns = Non-significant. *P<0.05 and **P<0.01 compared to control (ANOVA followed by Dunnett's test).

Table 2: Diuretic effect of various fruit extracts of *G. arborea* fruit extracts.

| Groups | Drug | Dose | Urine volume (ml/100g/5h) (X±SD) | Diuretic index (T/C) | Lipschitz value (Urine) (T/U) | Urine pH (X±SD) |
|---------------------|-----------------------------|-----------|----------------------------------|----------------------|-------------------------------|-----------------|
| I | NSW (C) | 2 ml/kg | 3.43±1.24 | - | -- | 7.09±0.78 |
| II | Urea (U) | 1 g/kg | 4.61±1.16* | 1.344 | -- | 6.75±0.81 |
| III | Ethanol extract (T) | 300 mg/kg | 5.74±1.07* | 1.673 | 1.245 | 6.69±0.66 |
| IV | Ethyl acetate extract (T) | 300 mg/kg | 5.04±1.14* | 1.469 | 1.093 | 6.76±0.91 |
| V | n-butanol extract (T) | 300 mg/kg | 6.17±1.21** | 1.798 | 1.338 | 6.79±0.49 |
| VI | Petroleum ether extract (T) | 300 mg/kg | 4.89±1.08* | 1.426 | 1.061 | 6.78±0.72 |
| ANOVA | | | | | | |
| Source of Variation | SS | df | MS | F | P-value | F crit |
| Between Groups | 10.0467 | 1 | 10.0467 | 2.62906 | 0.00091 | 4.9646 |
| Within Groups | 4.645 | 10 | 0.4645 | | | |
| Total | 14.6917 | 11 | | | | |

NSW – Normal saline water. Data are presented in mean ± standard deviation, n = 3. Standard error of mean < 0.716. ns = Non-significant. *P<0.05 and **P<0.01 compared to control (ANOVA followed by Dunnett's test).

In the control and standard groups, the excretion of sodium in urine for 5 h was found to be 109.6±1.25 and 121.34±1.08 mmol/l respectively. The excretion of sodium in urine for 5 h was 126.43±1.11, 124.22±1.21, 133.62±1.09 and 122.07±0.98 mmol/l for ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively. The dose at 300 mg/Kg of the extracts produced a significant increase in Na⁺ and K⁺ excretion, compared with the control group. In the control and standard groups, the excretion of potassium in urine for 5 h was found to be 67.82±1.17 and 77.61±1.05 mmol/l respectively. The excretion of sodium in urine for 5 h was 81.09±1.19, 79.23±1.21, 84.32±1.02 and 78.76±1.13 mmol/l for ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively. The normal value for Na⁺/K⁺ ratio is reported to be 1.551 to 1.616. The concentration of aldosterone is considered to be dependent on Na⁺/K⁺ ratio. If the Na⁺/K⁺ ratio falls below the normal value in plasma, the aldosterone secretion will be decreased and if the ratio rises above the normal value the aldosterone secretion will be increased. Significant increase in Na⁺, and K⁺ excretion was observed with all extracts treated animals but it was more than the standard (Urea) control. The volume of urine collected at the end of 5 h from treated animals was found to be 3.43±1.24, 4.61±1.16, 5.74±1.07, 5.04±1.14, 6.17±1.21 and 4.89±1.08 ml for control, standard, ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively. The pH of urine collected from treated animals was found in the range of 6.69±0.66 to 7.09±0.78. No change of pH in urine was found for standard drug as well as the four extracts. The results revealed that all fruit extracts of *G. arborea* have possessed significant diuretic activity and are comparable to both normal and standard (Urea) control. Among all the extracts, n-butanol showed good diuretic activity. It may be suggested that the fruits constituents might be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically. Previous studies have also demonstrated that there are several compounds which could be responsible for the plants diuretic effects such as flavonoids, saponins or organic acids².

The diuretic effect may be produced by stimulation of regional blood flow or initial vasodilatation, or by producing inhibition of tubular reabsorption of water and anions, the result in both cases being diuresis^{13,14}. The increased sodium and water excretion activity also provides strong basis for its proved anti-hypertensive action¹⁵.

CONCLUSION

It can be concluded that the extracts of *G. arborea* possess diuretic activity. However, the components responsible for the diuretic activity are currently unclear. Therefore, further investigation is needed to isolate and identify the compounds present in the fruit extracts which are responsible for exhibiting these therapeutic activities.

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