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Research Article

EFFECT OF AQUEOUS EXTRACT OF SEEDS OF *LAGENARIA SICERARIA* ON URINE VOLUME AND URINARY ELECTROLYTES IN SWISS ALBINO RATS

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ABSTRACT
The present study was to see the effect of aqueous extract of seeds of *Lagenaria siceraria* (AELSS) on urine volume and electrolytes in swiss albino rats and to compare with standard drug Hydrochlorothiazide. Thirty male Swiss albino rats aged ten to twelve weeks of male sex weighing about 200-225 g were taken, and after acute toxicity studies two different doses were selected. The animals were divided into five different groups. The first group was kept as the control (Normal Saline) group I, second as the standard (Hydrochlorothiazide) group II and the remaining two groups as Test III and Test IV, and given different doses of the AELSS. Diuretic and Natriuretic activities were carried out by administration of normal saline along with the treatment modules. The volume of urine (in ml) and the Na^+, K^+ and Cl^- content in the urine were measured. The extract at 100 and 200 mg/kg, produced significant (P-values < 0.05) Saluretic, Natriuretic, increased potassium elimination and decreased urinary volume. Thus the study elucidates that aqueous extract of seeds of *Lagenaria siceraria* possess significant aquatic, Saluretic, Natriuretic activity.
Keywords: Aquatic, Diuresis, *Lagenaria siceraria*, metabolic cages, Natriuretic and Saluretic.

INTRODUCTION

The plant, *Lagenaria siceraria* (Mol.) Standl. (Family: Cucurbitaceae), commonly known as bottle gourd, a common fruit vegetable used throughout the India. The gourd family consists of a number of bioactive plants, used extensively from ancient time for their therapeutic values. The fruit is used as diuretic, cardio-tonic, cardio-protective and nutritive agent. The fruit is a good source of vitamin B complex and choline and also source of vitamin C and β-carotene. It also contains Cucurbitacins, fibers and polyphenols[1-4]. *Lagenaria siceraria* belongs to Gourd family and is traditionally used for treatment of various disorders from a very long time as immunosuppressant[5], diuretic[6], cardio-tonic, cardio-protective[7] and nutritive agent.[8] The fruit is also reported to have good source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene.[9] HPLC analysis of methanolic extract from plant shows the presence of flavone-C glycosides[10]. Sterols namely campesterol and sitosterol have been identified and isolated from *Lagenaria siceraria* fruits, reported to possess antihepatotoxic activity[11]. The fruit has been reported to possess antioxidant activity[12], hypolipidemic and triton-induced hypolipidemic rats[13]. HPLC analysis of methanol extract from plant shows the presence of flavones-c glycosides[14]. Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of *Lagenaria siceraria* possesses immunoprotective, antitumor, anti HIV and antiproliferative properties[15]. Literature survey revealed that the plant fruit extract has been screened for its traditional diuretic activity in experimental animals but not the seed extract of the fruit of *Lagenaria siceraria*. Therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of seed of the fruit of the plant, *Lagenaria siceraria* as diuretic as its fruit and leaves.

MATERIALS AND METHODS

The study was done during June 2012 to March 2013. The seeds of *Lagenaria siceraria* were obtained from an authenticated Ayurvedic shop in Khammam, A.P, India.

Extraction Procedure

The preparation of extract from the seeds of *Lagenaria siceraria* was done in the department of Pharmacology, Mamata Medical College at Khammam, A.P, India. The seeds were finely powdered in a grinder. Aqueous extraction of the powdered material was done by soxhelts apparatus. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight.

Animals

Adult male Swiss albino rats, weighing between 200-225 g were used in the study. The animals were given free access to food and water. The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC) Registration number 285/CPCSEA, Lr. No. IAEC/DP-08/C32. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Drugs

Tab. Hydrochlorothiazide 25 mg, manufactured by Sun Pharmaceuticals was used in the study.

Toxicity evaluation in Albino rats

The aqueous extract was tested for its acute toxicity in albino rats. Acute oral toxicity was performed as per OECD-423 guide lines[16] to determine the acute toxicity, the extract was administered orally in an ascending order and in widely spaced doses that is 0.25 g/kg, 0.5 g/kg, 0.75 g/kg and 1 g/kg

to different groups of albino rats. Two albino rats were used in each group; the control albino rats received normal saline. The animals were observed periodically for forty eight hours. The parameters which were observed were hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions. There were no toxic effects and mortality. The optimization of the effective dose was calculated by taking one tenth of the maximum dose, that is 100 mg/kg and the other dose taken was double of the first dose, which is 200 mg/kg. These doses were then compared with the control group which received normal saline 25 ml/kg body weight and with the standard group which received hydrochlorothiazide 2.5 mg/kg body weight for the evaluation of the diuretic activity.

Experimental Design

The diuretic activity in rats was studied by Lipchitz test[17]. Adult male Swiss albino rats weighing between 200-225 g were used. The room temperature was maintained between 27-29^{o}C. Food was restricted 18 hours prior to the experiment with free access to water. All the animals were hydrated with 25 ml/kg of 0.9 % normal saline orally. The animals were divided into four groups with six rats in each group. In all the animals' urinary bladder was emptied before administration of drug. First group of six rats were kept as control, which were given only 0.9 % normal saline 25 ml/kg body weight orally. The animals were then transferred to the metabolic cages; three animals per cage and time noted. Second group of six rats were fed with normal saline 25 ml/kg along with standard hydrochlorothiazide 2.5 mg/kg orally and then transferred to the metabolic cages housing three animals per cage and time noted. The third and fourth group of six rats was taken as test groups and the aqueous extract of AELSS which was given orally along with normal saline at the dose of 100 mg/kg and 200 mg/kg keeping the volume administered constant. Animals were subsequently transferred to metabolic cages housing, three animals per cage. The urine was collected in beakers for a period of five hours in all groups. The rats were not given food or water during the experiment. At the end of five hours, the bladder of each rat was emptied by pulling the tail at the base to collect the residual urine. Urinary volume and urinary pH was noted and samples were taken for estimation of urinary electrolytes for sodium, potassium and chloride using spectrophotometer. The rats were kept back again in metabolic cages and urine after twenty four hours could not be collected as there was no urine collection. The experiment was done in triplicate to confirm urinary volume.

Measurement of Urinary volume and Electrolytes

The collected urine was estimated for volume. The pH was measured by using a digital pH meter. The pH reading was noted for the control, standard (hydrochlorothiazide) and different doses of test animals. The estimation of the urinary electrolytes was done by using a digital spectrophotometer (Mfd by Electronics India, Model 301) by using an electrolyte kit which was manufactured by M/S Excel Diagnostics, Pvt. Ltd, and Hyderabad, India.

Saluretic, Natriuretic and Carbonic Anhydrase Inhibition

The sum of Na^+ and Cl^- excretion was calculated as a parameter of Saluretic activity. The ratio Na^+/ K^+ was calculated for Natriuretic activity. The ratio $Cl^- /Na^+ + K^+$ (ion quotient) was calculated to estimate carbonic anhydrase inhibition[18].

Statistical analysis

The results are expressed as mean values ± S.D (standard Deviation) Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Dunnette's Multiple Comparisons Test. The results were considered statistically significant when P was < 0.05.

RESULTS

The urinary volume with Group I (control) and Group II (standard) was 6.23 ± 0.56 and 13.37 ± 0.95 where as with Group III (AELSS 100 mg/kg) and Group IV (AELSS 200 mg/kg) it was 2.93 ± 0.1 and 1.75 ± 0.15 when compared with control as shown in Table 1. Analysis of 5 h urinary excretion showed the urinary electrolyte contents with Group I (control) was, Na^+ 77.5 ± 1.37, K^+ 20.85 ± 0.85 and Cl^- 135.30 ± 1.75 and with Group II (standard) was Na^+ -168.4 ± 3.39, K^+-16 ± 0.62 and Cl^- - 147.46 ± 5.79. The urinary electrolyte content following the administration of the extracts, there was significant dose dependent increase in Na^+, K^+ and Cl^-excretion in two test groups that is in Group III (AELSS 100 mg/kg) 105.5 ± 3.54, 23.5 ± 1.5 and 295.65 ± 1.74 respectively and in Group IV (AELSS 200 mg/kg) Na^+, K^+ and Cl^- excretion was 108 ± 1.04, 24.05 ± 0.35 and 347.4 ± 0.64 respectively when compared with control group. Changes in parameters like pH were not significant. When compared with controlled group, both the doses of AELSS exhibited dose dependent increase in the excretion of electrolytes Na^+ K^+ and Cl^- and decrease in volume of urine excretion which indicates aquatic action of the extract as shown in Table 1 and the data also indicate that AELSS extract in tested doses produced significant and dose-dependent Saluretic and Natriuretic activity without carbonic anhydrase enzyme inhibition activity as shown in Table 2.

Evaluation

The sum of Na^+ and Cl^- excretion is calculated as Parameter for Saluretic activity. The ratio Na^+/K^+ is calculated for Natriuretic activity. Values greater than 2.0 indicate a favorable Natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect. The ratio $Cl^-/Na^+ + K^+$ (ion quotient) is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

DISCUSSION

Even though this is modern era of synthetic drugs, still plants are indispensable source of medicinal preparations even today. Ancient Indians had a rich knowledge of the use of medicinal plants in various diseases. The Materia Medica of Ayurveda and Unani systems contains a rich heritage of indigenous herbal drugs[19]. A right scientific and systematic approach is needed for biological evaluation of plant products, based on their use in traditional medicine for the development of new drug molecules from plants. *Lagenaria siceraria* (Molina) (Family: Cucurbitaceae) is one of them. It is a large, softly pubescent, annular, climbing herb which grows throughout the India.[20] Different parts of plant *Lagenaria siceraria* has been studied extensively for various disorders. LS juice prevents excessive loss of sodium, satiating thirst, and giving a cooling effect.[21] The root is applied in the treatment of dropsy.[22] The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic, antibilious,

and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness.[21-23] The seeds contain steroidal moieties like avenasterol, codisterol, elesterol, isofucasterol, stigmasterol, sitosterol, compesterol, spinasterol; and sugar moieties including rhamnose, fructose, glucose, sucrose, raffinose[24] and saponin.[21,25] Seed kernels are rich in iron, potassium, sulfur, and magnesium and particularly rich in copper (28.3 ppm); they can be used as a dietary supplement.[26] *L. siceraria* seeds are used in migraine type headache and pain and are reported to contain saponins, essential fixed oils, vitamins[27,28] Lagenin – a novel ribosome inactivating protein has been isolated from the lyophilized water extract of seeds which is known to possess immunosuppressive, antitumor, antiviral, antiproliferative and anti-HIV activities[15] Diuretic effect of different parts of, *Lagenaria siceraria* fruit, leaf is studied in recent years for their diuretic activity but not seeds of *Lagenaria siceraria* fruit. In one study the elevated diuretic potentials of juice and methanol extract were statistically significant and comparable to that of the standard diuretic agent furosemide (20 mg/kg; i.p.).[29] Our study was to evaluate the diuretic activity of seeds of *Lagenaria siceraria* fruit. The results obtained in this study indicate that AELSS has effective hypernatremia, hyerchloremic and hyperkalaemic Saluretic action. (Increased Na^+, K^+ and Cl^- excretion) but the result showed dose dependent decrease in urinary volume excretion. This finding was confirmed by repeating the experiment three times. Data indicate that AELSS extract in tested doses produced significant and dose-dependent aquatic and Saluretic and Natriuretic activity without carbonic anhydrase enzyme inhibition activity as shown in Table 1 and 2. Diuretics are drugs that bring about an increase in urinary volume as well as in the electrolyte output. Due to this, they are used to regulate both volume and composition of the urine in different affections like high blood pressure, heart failure and nephritic syndromes[30]. As the whole fruit of *Lagenaria siceraria* has scientifically proven diuretic activity but not the seeds of the *Lagenaria siceraria* fruit, prompted us to see weather seed has also has diuretic activity. Our result shows that the seed extract has aquatic and significant Saluretic action that means the extract has ADH/Vasopressin like action. Vasopressin, also known as arginine vasopressin (AVP), antidiuretic hormone (ADH), or argipressin, is a neurohypophysial hormone found in most mammals. Its two primary functions are to retain water in the body and to constrict blood vessels. Vasopressin regulates the body's retention of water by acting to increase water reabsorption in the collecting ducts of the kidney nephron.[31,32] Vasopressin is a peptide hormone that increases water permeability of the kidney's collecting duct and distal convoluted tubule by inducing translocation of aquaporin-CD water channels in the kidney nephron collecting duct plasma membrane.[33] It also increases peripheral vascular resistance, which in turn increases arterial blood pressure. It plays a key role in homeostasis, by the regulation of water, glucose, and salts in the blood. It is derived from a preprohormone precursor that is synthesized in the hypothalamus and stored in vesicles at the pituitary. It has a very short half-life between 16-24 minutes.[34] Diabetes insipidus is a condition in which our bodies ability to control the balance of water within our body is not working properly. As mentioned above, ADH usually helps kidneys to concentrate urine. If less ADH is released, we will pass an increased volume of dilute urine. The levels of sodium and potassium salts in our blood can be high in diabetes insipidus. Treatment of Diabetes insipidus is replacement with ADH which is lacking. This study results shows that the seed extract has aquatic (inducing translocation of aquaporin-CD water channels) and significant Saluretic action that means the extract has ADH/Vasopressin like action. In addition the extract might have Na^+Cl^- symport blocking action like Hydrochlorthiazide on early distal convoluted tubule. Further studies are to be conducted to elucidate above said actions and can be used in clinical conditions associated with hypernatremia, hyperkalaemia and hyperchloreamia.

Table 1: Effect of *Lagenaria siceraria* seed extracts on urine volume, ph, sodium, and potassium and chloride excretion

Groups (n = 6)	Urinary Ph	Urinary volume ml/kg	Urinary Na^+ meq/kg	Urinary K^+ meq/kg	Urinary Cl^- meq/kg
Group-I Control NS 25 ml/kg,	7.4 ± 0.12	6.23 ± 0.56	77.5 ± 1.37	20.85 ± 0.85	135.30 ± 1.75
Group-II Standard HZ 25 mg/kg	7.12 ± 0.12	13.37 ± 0.95	168.4 ± 3.39	16 ± 0.62	147.46 ± 5.79
Group-III Test-I (AELSS) 100 mg/kg	8.31 ± 0.27	2.93 ± 0.1*	105.5 ± 3.54*	23.5 ± 1.5*	295.65 ± 1.74*
Group-IV Test-II (AELSS) 200 mg/kg	9.03 ± 0.16	1.75 ± 0.15*	108 ± 1.04*	24.05 ± 0.35*	347.4 ± 0.64*

AELSS- Aqueous extract of *Lagenaria siceraria* Seed, HT-Hydrochlorothiazide, n = 6, Mean ± SD, *<0.05

Table 2: Effect of *Lagenaria siceraria* Seed extract on Saluretic, Natriuretic and Carbonic anhydrase inhibition

Groups (n = 6)	Saluretic activity	Natriuretic activity	Carbonic anhydrase inhibition
Group-I Control NS 25 ml/kg,	212.8	3.72	1.37
Group-II Standard HZ 25 mg/kg	315.8	10.5	0.79
Group-III Test-I (AELSS) 100 mg/kg	401	4.4	2.28
Group-IV Test-II (AELSS) 200 mg/kg	455	4.5	2.62

AELSS- Aqueous extract of *Lagenaria siceraria* Seed, HT-Hydrochlorothiazide

CONCLUSION

Diuretic effect of different parts of, *Lagenaria siceraria* fruit, leaf is studied in recent years for their diuretic activity but not seeds of *Lagenaria siceraria* fruit. The results obtained in this study confirmed that seed extract of *Lagenaria siceraria* fruit has potent Saluretic, Natriuretic, and hyperkaliuretic action along with aquatic (Inducing translocation of aquaporin-CD water channels) action without carbonic anhydrase inhibition action. Chemically, *Lagenaria siceraria* contains various biologically active phytoconstituents including flavonoids, saponins, triterpenes, and volatile

principles. The antidiuretic action may be due the presence of those bioactive compounds.

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