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Role of *Salacia lehmbachii* extract in renal function

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Abstract

Purpose: To assess the possible role of aqueous root extract of *Salacia lehmbachii* (ARESL) in renal function of Wistar rats. Materials and Methods: Healthy rats were randomized into 5 groups of 6 animals and administered with 25 ml/kg saline. Group I (control) received saline (10 ml/kg), Group II (standard) received urea (1 g/kg), Groups III and V (tests) received ARESL (1 and 2 g/kg) respectively and Group V received urea plus ARESL (1:2 g/kg) by oral gavage. Urine pH, volume, conductivity and electrolyte (Na^+, K^+ and Cl^-) concentrations were evaluated using modified Lipschitzs method. Results: Preliminary phytochemical analysis of extract revealed the presence of alkaloids, glycosides, saponins, flavonoids and anthraquinones in varied considerable quantities. Administration of the ARESLled to increase in Na^+ and K^+ level without altering Na^+ / K^+ and Cl^- / Na^+ + K^+ ratio and non-significantly increased the pH. Renal toxicity or any adverse effect was not observed in the course of the study. Conclusion: The present study reveals that extract of *S. lehmbachii* conserves significantly fluid in tested rat model and supports the pharmacological credence to the folkloric medical usage.

INTRODUCTION

Renal function is an indication of the state of the kidney and its role in renal physiology. Renal physiology is often altered during disease situation necessitating the control of electrolytes through diuretic or anti-diuretic agents. Renal physiology in some disease states like congestive heart failure, nephritic syndrome, liver cirrhosis, renal failure, hypertension and pregnancy toxaemia is potentiated by diuretic agents to increase the rate of urine flow and sodium excretion [1-4]. Whereas in clinical disease situation like polyuria, anti-diuretic drugs are used to decrease the rate of urine flow and sodium excretion. In either case these agents have the potency of producing adverse side effects including hypo/hyperkalemia, hypo/hypervolemia, hypo/hypernatraemia, hypo/hypercalaemia, hypo/hyperuricaemia and metabolic disorders [5-8]. Hence, a search for phyto-renal-tolerant agents that retain therapeutic efficacy with little or no side effects is justified. *Salacia lehmbachii* belongs to the family celestraceae [9]. It is a small woody tree of about 3 m height. The leaves are simple, ovate oblong, opposite, acuminate and shining. The flowers are crystal yellow or orange borne on woody auxiliary tubercles. The fruits are globose, orange and contain one large seed at the centre and two-four seeds immersed almost at the periphery of the pulp [10]. *S. lehmbachii* is commonly found in the tropical forest of Cameroon and South Eastern Nigeria [11]. It has been reported to possess analgesic and anti-inflammatory potentials [12] and anti-abortificient activities in rat model [13]. South Eastern Nigerian herbalists use the root in treating diseases including polyuria, malaria fever and threatened abortion with no scientific backup. Consequently, the present study was aimed at assessing effect of ARESL in renal function of Wistar rat model since there is great similarity and homology between the genomes of humans and rodents [14].

MATERIALS AND METHODS

Plant material and preparation of extract

Fresh root of *Salacia lehmbachii* was harvested from Uruk Otong village of Ukanafun Local Government Area of Akwa Ibom State, Nigeria, in March 2013. The whole plant was identified in Cameroon National Herbarium (CNH), Yaounde, with Voucher No. 40730/SRF/CAM. The roots were dried at room temperature (25-30°C) for 3 weeks. The dried root sample was then ground to coarse powder using manual grinding machine and preserved in air tight container. The dried and ground root material (1 kg) was macerated fast in petroleum ether (5 litres) to remove the fatty component of the extract and secondly macerated in distilled water (8 litres) at room temperature for 3 days, filtered and sun-dried at 40 ± 4^{O}C for another 3 days to obtain a chocolate-like paste extract with a yield of 6.7%.

Experimental animals

Thirty healthy male Wistar rats weighing between 180-200 g were randomly selected from Animal House Unit, Department of Pharmacology, University of Calabar and used for the experiment. The animals were housed in polyvinyl cages of at least 4 animals per cage and maintained under standard laboratory conditions of temperature (28 ± 2^{0}C), relative humidity (50 ± 5%), a 12 hour (h) dark/light cycle and received standard pellet diet and water *ad libitum*. To keep the hydration rate constant, food and water were stopped 12 h before the experiments. This animal experimentation was carried out following the guidelines of the CPCSEA [15].

Phytochemical screening

ARESL was qualitatively screened for the presence of phytoactive constituents such as alkaloids, glycosides, flavonoids, tannins, saponins, polyphenols and glycosides following standard tests procedures [16].

Acute toxicity study

The acute toxicity study was carried out in adult female albino rats by modified 'Up and Down' procedure [17]. Briefly, 10 female albino rats were divided into 2 groups of 5 rats each. The animals were fasted overnight and next day ARESL dissolved in distilled water was administered orally as a single dose at different dose levels. Then, the animals were observed for general behavioural, neurological and autonomic profiles for the next 3 h and finally after 24 h [18].

Experimental procedure

The method of Lipschitz *et al* [19] was employed with light modifications. Eighteen hours fasted Wistar rats with no access to drinking water were randomized into 5 groups of 6 rats and administered 25 ml/kg saline to impose uniform water load. Group I (control) received saline (10 ml/kg), Group II (standard) received urea (1 g/kg), Groups III and V received ARESL (1 and 2 g/kg) respectively and Group V received urea plus ARESL (1: 2 g/kg) by oral gavage. The animals were placed in metabolic cages provided with a wire mesh bottom and a funnel for collecting urine. Stainless steel sieves were placed in the funnel to retain the faeces while allowing only urine to flow down for collection and measurement. Three rats were placed in one metabolic cage and urine excretion was recorded after 5 h. Sodium and potassium contents of the collected urine were estimated by Flame Photometer (Jenway, model PFP7). 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 (metrohm 712 Toshniwal group model TCM-15) and pH was measured with a digital pH meter (MK-VI, Unique instruments & machineries, Calcutta) on fresh urine sample.

Statistical analysis

Data were expressed as means ± SEM (n = 6) and were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey test as post hoc. Values of $P < 0.05$ were considered statistically significant [20].

RESULTS

Screening assays to determine bioactive constituents of aqueous root extract of *S. lehmbachii* are presented in Table. 1 with alkaloids, saponins and tannins being highly present. Assessment of effect of aqueous root extract of *S. lehmbachii* in urine production by kidneys per 100 g body weight of rat is presented in Fig. 1. After 3 h of drug/extract administration of the rats, urine production was only evident in control group which was treated with 10 ml/kg saline. Between 3-9 h of administration, urine production was significantly ($P < 0.05$ for standard group and $P < 0.01$ for groups III-V) inhibited as compared to control group. Between 9-12 h, only group II (standard) and

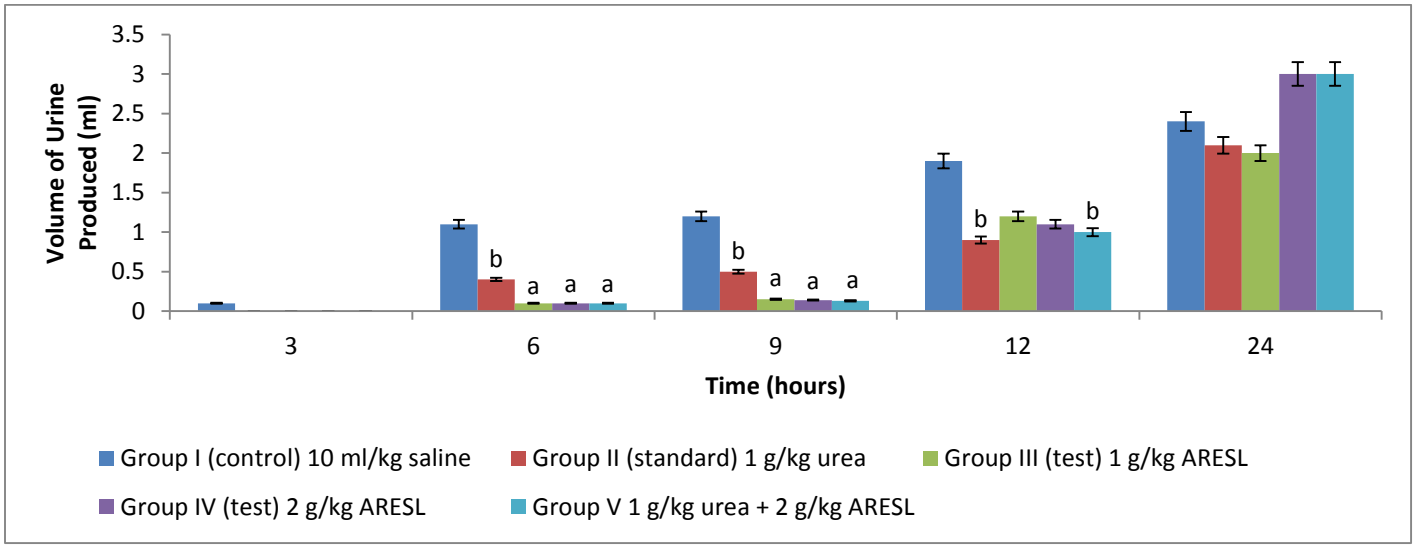
group V showed significant (P <0.05) conservation of body fluid. Between 12-24 h, there was no more significant change in volume of urine produced.

Table. 1: Preliminary phytochemical composition of aqueous root extract of *Salacia lehmbachii*

Bioactive molecules	
Alkaloids	+++
Glycosides	++
Saponins	+++
Tannins	+++
Flavonoids	++
Reducing compounds	+
Phenols	++
Anthraquinones	++
Phlobatannins	-

+++ = highly present, ++ = moderately present, + = lowly present

Fig. 1: Effect of ARESL in urine production per 100 g body weight of rat



DATA is presented as Mean ± SEM (n = 6). [a]P <0.01, [b]P <0.05

Table 2. Effect of ARESL on electrolyte concentrations, pH and conductivity after 24 h in rat urine primed with 25 ml/kg saline

Treatment	Dose (g/kg)	Electrolyte concentrations (meq/l)					pH	Conductivity (µS)
		Na^+	K^+	Cl^-	Na^+/K^+ ratio	$Cl^-/(Na^+ + K^+)$		
Group 1 (control) saline	10 ml/kg	5848.67±584.20	2914.01±321.20	868.31±47.25	2.00±1.88	0.10±0.05	6.61±0.25	18.01±2.02
Group II (standard) urea	1	8976.36±673.63*	7696.21±658.06*	1732.88±84.97*	1.17±1.02	0.10±0.06	6.92±0.21	29.54±3.41*
Group IV (test) ARESL	1	6374.31±572.05	4983.23±452.14*	1398.21±72.73*	1.23±1.27	0.12±0.07	6.70±0.20	21.34±2.38
Group V (test) ARESL	2	8272.35±642.08*	6582.51±657.10*	1543.63±82.32*	1.26±0.98	0.90±0.06	6.73±0.24	27.68±3.21*

Group VI urea + ARESL	1: 2	8862.36±662.08*	6834.35±655.16*	1642.62±82.41*	1.30±1.01	0.11±0.06	6.75±0.27	28.77±3.39*

Data is presented as Mean ± SEM (n = 6),*P <0.05

The effect of ARESL on electrolytes concentrations, pH and conductivity after 24 h on rat urine primed with 25 ml/kg saline is presented in Table 1. All groups showed significant difference (P <0.05) in Na^+, K^+ and Cl^- secretion though no significance difference was seen in Na^+/ K^+ and $Cl^-/ Na^+ + K^+$ ratio as compared to control group. There was no significant change in the pH of urine obtained from all groups. However, conductivity of urine obtained from groups treated with ARESL showed significant change (P <0.05).

DISCUSSION

Urea is important for the conservation of body water due to its role in the production of concentrated urine in the renal inner medulla. The passive mechanism hypothesis for urine concentration in the inner medulla requires the delivery of large quantities of urea to the deepest portions of the inner medullary (papillary) Interstitium [21-22]. This is achieved through the transport of urea via vasopressin-regulated urea transporter (UT-A1) in terminal inner medullary collecting duct (IMCD) [23-24]. In addition, the erythrocyte urea transporter (UT-B) minimizes the loss of urea from the inner medulla by increasing the efficiency of countercurrent exchange for urea in the vasa recta [25]. Thus, the production of concentrated urine and the conservation of body water depend on urea transporters in both IMCD and erythrocytes. In the present study, ARESL at concentrations of 1 g/kg and 2 g/kg body weights conserved significantly body fluid between 0-12 h of administration as compared to control. This suggests that the extract has bioactive molecules that mimic urea and therefore are transported through UT-A1 in terminal IMCD. Jouad *et al* [26] reported that water extract of *Spergularia purpurea* administered to normal rats resulted in increased urine production through increase in glomerular filtration rate (GFR) due to either an increase in arterial pressure/glomerular blood flow or by decreasing renal perfusion pressure. In the present study, extract of *S. lehmbachii* appeared to inhibit regional blood flow through inhibition of prostaglandin on smooth muscle cells resulting in less urine production and conservation of body fluid. The extract could have also acted through mesangial cells in glomerulus of kidney to decrease GFR. Further from 12-24 h, there was increase in urine production suggesting that the half-life of the extract should lie within these hours. The collecting duct has principal cell which mediates its influence on sodium and potassium balance through sodium channels and potassium channels located on the cell's apical membrane. Aldosterone determines expression of sodium channels with increased aldosterone causing increased expression of luminal sodium channels [27]. Aldosterone increases the number of Na^+/K^+-ATPase pumps that allow increased sodium reabsorption and potassium secretion. Vasopressin determines the expression of aquaporin channels on the cell surface [28, 29]. In the present study, ARESL increased significantly the secretion of Na^+ and K^+ suggesting that the number of Na^+/K^+-ATPase pumps were desensitized together with aquaporin channels from possible inhibition of adolsterone synthesis by adrenal gland and vasopressin secretion by posterior pituitary. In 1958, Broadbent and Schnieden implicated dioscorine and dioscine alkaloids in anti-diuretic activity [30] while more recently, Choudhury *et al* has implicated chrysin, oroxylin-A, baicalein, biochanin-A and ellagic acid flavonoids in anti-diuretic activity [31]. It is also known that some plant (*Rubia tinctorium* L) anthraquinone derivates have been used as anti-diuretic drugs [32]. In the present study, preliminary phytochemical screening of ARESL revealed the presence of alkaloids, flavonoids, anthraquinones which undoubtedly have laid credence to water retention ability of the extract.

CONCLUSION

Thus, aqueous root extract of *Salacia lehmbachii* possesses a powerful natriuretic and not aquaretic property. This adds to the extract's analgesic and anti-inflammatory, anti-abortificient properties evaluated by us much earlier [12-13]. Based on these researches, *S.*

lehmbachii is good candidate plant for in-depth research into obtaining phytotherapeutic agents with relatively large therapeutic window and efficacy in these disorders.

COMPETING INTERESTS

Authors declare that there is no conflict of interests regarding the publication of this research work.

AUTHORS' CONTRIBUTIONS

Author ADE designed the study and wrote the protocol. Author LPT wrote the first draft of the manuscript and performed the statistical analysis. Author PMU managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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