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Pharmacological investigation on the diuretic activity of the aqueous fruit extract of *Neolamarckia cadamba* (Roxb) Bosser

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ABSTRACT

The study was formulated to evaluate the efficacy of aqueous fruit extract of *Neolamarckia cadamba* on diuretic property in albino rats. Adult albino rats were randomly divided into five groups (n=8). All groups received normal saline (25 ml/kg). Group I which served as control received normal saline and animals which received standard diuretic drug furosemide (750mg/kg) were taken as diuretic control (group 2). Group 3 and group 4 received the fruit extract at a dose of 200 mg/kg and 400 mg/kg body weight. 24 h urine samples were collected by keeping the animals in metabolic cages. After 24 hrs of experiment, blood samples were collected from tail vein and serum was analyzed for creatinine concentration. Urine samples were analyzed for volume, pH, conductivity, urinary excretion, electrolyte concentration (Na^+, K^+, Cl^-), Ca^{2+}, creatinine, creatinine clearance, glomerular filtration rate (GFR), saluretic, natriuretic and diuretic activity. The results were expressed in mean ± SE and analyzed with one way ANOVA. $P< 0.05$ was considered statistically significant. Significant dose dependant increase in serum creatinine, urine creatinine, creatinine clearance, glomerular filtration rate, saluretic and natriuretic activity was observed in the fruit extract treated groups. Urine volume, urinary excretion, electrolyte concentration and Ca^{2+} were also increased in the fruit extract treated groups when compared with controls. At a dose of 200mg/kg extract had moderate diuretic activity and a higher dose of 400mg/kg had good diuretic activity. The present study confirmed the effectiveness of aqueous fruit extract of *N. cadamba* as diuretic agent.

Key-words: *Neolamarckia cadamba*, diuretic activity, furosemide, glomerular filtration rate

INTRODUCTION

Neolamarckia cadamba is a fast growing large-sized ornamental and shade living deciduous evergreen tree belonging to the family Rubiaceae[1]. It is commonly known as 'kadam' in India, 'Luran' in Malyasia and 'Jabon' in Indonesia[2] and is considered a sacred tree in India. The tree is found mostly in the warm forests native to south and Southeast Asia. A fully mature kadamba tree can reach up to 45 m in height. It is a large tree with a broad crown and straight cylindrical bole. It is quick growing, with broad spreading branches and rapid growth in the first 6–8 years. The kadamba flowers are sweetly fragrant, red to orange in colour, occurring in dense, globular heads of approximately 5.5 cm diameter. The fruits are small, round balls with fleshy capsules packed closely together to form a fleshy yellow – orange inflorescence. This contains approximately 8000 sweet and edible seeds. On maturing, the fruit splits apart and releases the seeds, which are then dispersed by wind or rain[3]. The excessive thirst during fevers is often quenched with its fruit juice. The tree is valued for its fragrance, the flowers being used to make perfume. Externally, wounds and ulcers are dressed with its slightly warmed leaves to alleviate the pain, swelling, cleansing and better healing of wounds.

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Medically kadamba is more important for ailments associated with blood, edema, cough, uterine complaints, diarrhea, dysentery and colitis[4]. Kadamba is useful against skin diseases as it improves the complexion of the skin. The leaves are said to be nutritious, astringent and bitter; their decoction is reported to be used for gargling in apathies or stomatilies. Menorrhagia is effectively controlled with the fresh juice of its leaves or their decoction. The fruit juice augments the quantity of milk in lactating mothers. Antiurolithiatic potential of aqueous fruit extract[5] and antibacterial activity of leaf and bark are reported[6]. The decoction of roots is salutary in urinary ailments like dysuria, urinary calculi and glycosuria. The bark and leaves of the plant are reported to possess various medicinal properties, which makes it an astringent, anti-hepatotoxic[7], antidiuretic, antiseptic, antihelmintic, and wound healing abilities.

A glycosidic alkaloid, isodihydro cadambine, triterpenic acid and cadambagenic acid are isolated and characterized from kadamba. Cadambine and 3 – isodihydrocadambine are isolated as acetates from leaves. A polysaccharide composed of xylose, mannose and glucose in ratio 1:3:5 is isolated from seeds. The work on chemical composition of bark revealed the presence of saponin[8], alkaloids[9] and steroids[10]. Even though many studies have been conducted on this medicinal plant, no attempt has been made to evaluate the effect of fruit of *N. cadamba* (AFENC) on the diuretic activity and hence the present study has been formulated to evaluate the efficacy of fruit extract on diuretic property in mammalian model.

MATERIALS AND METHODS

Plant material

The fruits of *N. cadamba* were collected from the Kerala University campus, Karyavattom, Thiruvananthapuram ($8^0$37'36''N, $76^0$50'14''E), in Kerala state of India. Voucher specimen was kept in Department of Botany, University of Kerala, Kariavattom for further reference (Voucher no: KUBH 5811).

Preparation of the fruit extract

The collected fresh fruits were washed thoroughly, chopped into pieces and air dried at low temperature in the oven until the fruits become dry. Dried fruits were milled in a mechanical grinder to make it powder. The aqueous extract of the fruit was prepared by keeping the powdered plant material in soxhlet extraction apparatus for 74 hrs, using distilled water as solvent and was concentrated with rotary vacuum evaporator to separate the solvent. This concentrated fruit extract was refrigerated and administered to the experimental animals at specific doses.

Experimental animals

Healthy adult male albino rats of Wistar strain weighing 150–200 g were used for the diuretic activity. They were fed with standard rat chow diet and water *ad libitum*. The animals were housed in polypropylene cages maintained under standard environmental conditions (12-hr light/12-hr dark cycle; 25±3°C temperature, 35–60% relative humidity). All the animal experiments were conducted strictly according to the CPCSEA guidelines and the study was conducted after obtaining permission from Institutional Animal Ethics Committee (IAEC). (Permission number: - IAEC-KU-23/2011-12-ZOOL-GP (3)).

Acute toxicity study

Acute toxicity test of the aqueous extract of the fruit was determined using two groups of five Swiss albino mice of both sexes. After the administration of the extract, animals were observed for any gross behavioural changes. The extract was found to be safe up to 2000 mg/kg. 1/10 and 1/15 of the safe levels of extract was selected as effective doses for the experimental animals.

Chemicals

All chemicals used were of analytical grade and were purchased from Sisco Research Laboratory. Furosemide (LASIX) was used as a standard reference diuretic drug for the experimental purpose.

Assessment of diuretic activity

Pharmacological evaluation of diuretic activity

Adult healthy albino rats of Wistar stain with a weight of 150-200 g were randomly divided into five groups. Each group consists of eight animals. Animals in all the groups received normal saline (25 ml/kg) orally using gastric intubation tube. Prior to the experiment, animals were fasted overnight. Group 1 which served as the normal control received the normal saline. Animals which received the standard diuretic drug furosemide were taken as group 2. Group 3 and group 4 consisted of the fruit extract-treated group at a dose of 200 mg/kg (dose 1) and 400 mg/kg (dose 2) body weight respectively. After the administration of the treatments, animals were kept in metabolic cages, specially designed to separate urine and faeces. Animals were deprived of food and water during the urine collection period. 24 h urine samples were collected from the experimental animals in the measuring cylinder. After the 24 hrs of treatment period, blood samples were collected from the tail vein. Blood samples were centrifuged to separate the serum for measuring the serum creatinine level.

Analytical procedures

24 h urine samples were analysed for checking the urine volume, pH, conductivity, Na^+, K^+, Ca^+, PO_4 and chloride. Blood samples were centrifuged to separate the serum, which was used to determine serum creatinine to evaluate the kidney functioning. pH and conductivity were measured using standard digital pH meter and conductivity meter respectively. Sodium and potassium excretion rate was calculated with flame photometer using standard solutions to calibrate the instrument. Chloride content was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator. The other parameters such as urinary excretion, diuretic action, diuretic activity, saluretic and natriuretic activity were monitored for individual rats and were calculated using standard formulas.

Preliminary phytochemical analysis

The powdered fruit material in different extracts were subjected to preliminary phytochemical analysis to find out the active phytochemicals present in the fruit extract by using standard phytochemical tests.

Statistical analysis

The results were expressed as the mean± SE. Statistical analysis and comparison among different treatment groups were analysed using analysis of variance (ANOVA). The $p \leq 0.01$ and $p \leq 0.05$ were considered significant.

RESULTS

In the present investigation on diuretic activity of aqueous fruit extract of *N. cadamba* (AFENC), significant increase ($p < 0.01$) in urine volume was observed in the furosemide-treated group of rats than in the other treatment groups. Rats treated with AFENC at a dose of 400 mg/kg exhibited an increase in urine volume. But the values are not statistically significant. The pH values of all the extract-treated groups indicated that the urine is slightly alkaline in nature compared to the furosemide groups. Animals received the dose of 400 mg/kg exhibited a significant ($p < 0.05$) increase in pH value towards alkaline. Compared to the saline treatment groups the sodium excretion increased in all the other groups. Among the treatment groups, dose 2 group showed a significant increase ($p < 0.01$) in sodium excretion when compared to group 1 and group 2 animals. In the case of potassium excretion dose 2 group exhibited increased potassium excretion when compared to dose 1 group (**Table 1**).

Urinary calcium level increased in furosemide-treated animals than in those of the group 1. But the dose 1 group showed decreased excretion of calcium than the furosemide groups. Dose 2 group also exhibited a decrease in calcium excretion but the values were not statistically significant. When compared to the group 2, phosphate excretion increased in the rats receiving the dose of 200 mg/kg and 400 mg/kg body weight. Phosphate excretion rate showed a significant ($p < 0.01$) rise in dose 2-treated animals than that of dose 1-treated animals. Decreased level of conductivity was observed in the rats receiving the standard diuretic drug-treated animals, when compared to saline-loaded animals. Urine conductivity increased in both dose 1 and dose 2 groups. But animals receiving AFENC at the high dose showed increased urine conductivity when compared to group 1. When compared to standard diuretic drug, dose 1 ($p < 0.05$) and dose 2 ($p < 0.01$) groups exhibited significant increase in urine conductivity. Among the groups, dose 2-treated groups exhibited increased conductivity.

No significant variation in chloride excretion was observed in both doses of aqueous fruit extract of *N. cadamba* compared to the furosemide-treated rats. Animals received the high dose of *N. cadamba* had significant ($p < 0.05$) decrease from the saline-loaded groups. But the value is in the range of standard diuretic drug, furosemide. Creatinine excretion significantly increased in group 3 ($p < 0.05$) and group 4 ($p < 0.01$) compared to the saline treated and furosemide treated rats. A remarkable decrease in serum creatinine was noted in high dose ($p < 0.01$) and low dose ($p < 0.01$) groups compared with the group 2. Among the treatment groups, dose 2 group had significant ($p < 0.05$) reduction in serum creatinine in comparison with dose 1 group. A highly significant ($p < 0.05$) increase in glomerular filtration rate was recorded in dose 2-treated animals when compared with saline- and furosemide-treated animals (group 2).

Saluretic activity showed a remarkable increase in dose 1 and dose 2 groups. Among both group, dose 2 had significant increase in saluretic activity ($p < 0.05$) when compared with the standard drug furosemide. Highly significant ($p < 0.01$) increase in saluretic activity was observed in dose 2 animals than in the animals received dose 1. When compared with the saline groups, natriuretic values showed an increase in all experimental groups even though the values are not statistically significant. Compared with the standard diuretic drug, dose 1 and dose 2 exhibited remarkable increase in natriuretic activity but the values are not statistically significant. Urinary excretrion rate in dose 1-treated animals was found to be the same as in furosemide-treated groups. Rate of urinary excretion increased in animals which received 400 mg/kg body weight than in all other groups. The values of diuretic index significantly increased in 200 mg/kg ($p < 0.01$) and 400 mg/kg body weight ($p < 0.01$) when compared with group 2 animals. The diuretic activity was highly significant ($p < 0.01$) in rats receiving the AFENC at high dose (400 mg/kg). Dose 1 group also has significant ($p < 0.05$) diuretic activity than the animals of the group receiving the standard diuretic drug.

DISCUSSION

This study was carried out to determine the diuretic activity of AFENC in Wistar albino rats. The diuretic activity of the test drug was assessed with the standard diuretic drug, furosemide (LASIX), a high ceiling loop diuretic widely used as standard diuretic in clinical practices for comparing the pharmacological responses[11]. The results revealed that the fruit extract of *N. cadamba* increased the volume of urine output. When compared to the control groups, dose 2 groups have increased urine volume than in dose 1. From this it is evident that the extract has the capability to increase the volume of urine. It is one of the most important criteria for a diuretic. The diuretic effect may be produced by increased blood flow or vasodilation or by producing inhibition of tubular reabsorption of water and anions[12, 13]. Diuretics have two important characteristics such as the increased rate of urinary output and the decreased absorption of solutes. The mechanism behind this process is the inhibition of tubular reabsorption of solutes (electrolytes) and water into the blood stream, thereby increasing the urine output[11]. The urine conductivity is an indirect measure of ionic content of urine and this increased in both the extract-treated groups in a dose-dependent manner when compared to group 2. Electrolyte concentrations of sodium, chloride, potassium, calcium and phosphate in urine increased in the high dose group of the extract (400 mg/kg) than in the low dose treated group (200 mg/kg) when compared to the normal saline-treated animals (**Table 1**).

Table 1: Effect of oral administration of AFENC on urine volume, pH, conductivity and electrolyte concentration

Treatment	Dose mg/kg	Urine volume (ml)	Urine pH	Urine conductivity	Urinary electrolyte excretion Na+ (mEq/l)	K+ (mEq/l)	Cl- (mEq/l)
Group 1 Normal saline	25mg/kg	3.33±0.234	9.14±0.05	30±8.53	38.58±6.60	4.12±0.73	130±1.63
Group 2 Furosemide	750mg/kg	20.07±4.17^{a**}	8.89±0.07	36.5±1.89^{a**}	43.17±5.17	2.41±0.46	145±3.00
Group 3 AFENC	Dose1 (200mg/kg)	3.82±0.73	9.06±0.13	64.25±7.44 ^{b*}	71.88±9.27	3.79±0.92	138.5±2.63^{a**}
Group 4 AFENC	Dose 2 (400mg/kg)	6.7±1.078	9.24±0.05	72.75±3.71 ^{b**}	106.47±12.91^{a**b**}	4.65±0.31	141±1.29 ^{a**}

Values are expressed as mean ± SE for four animals in each group. One way Anova followed by Tukey test. a-indicates significant difference with normal control groups, b- indicates significant difference with diuretic control groups, c-indicates significant difference with dose I, d- indicates significant difference with dose II. *-P<0.05, **-P<0.01.

Table 2: Effect of oral administration of AFENC on urinary excretion, urine creatinine, serum creatinine, creatinine clearance and glomerular filtration rate (GFR)

Treatment	Dose mg/kg	Urinary excretion	Urine creatinine	Serum creatinine	Creatinine clearence	GFR
Group 1 Normal saline	25mg/kg	59.19±5.57	0.04±0.006	0.58±0.054	0.25±0.053	0.23±0.034
Group 2 Furosemide	750mg/kg	75.83±15.91	0.03±0.006	2.85±0.29^{a**}	0.27±0.016	0.22±0.013
Group 3 AFENC	Dose 1 (200mg/kg)	75.83±15.91	0.19±0.030^{a**b**}	1.53±0.16 ^{a**b**}	0.5±0.096	0.4±0.78
Group 4 AFENC	Dose 2 (400mg/kg)	106.62±16.43	0.169±0.046 ^{a*b*}	0.69±0.083 ^{b**c*}	2.5±0.580 ^{a**b**c**}	1.62±0.69 ^{a*b*}

Values are expressed as mean ± SE for four animals in each group. One way Anova followed by Tukey test. a-indicates significant difference with normal control groups, b- indicates significant difference with diuretic control groups, c-indicates significant difference with dose I, d- indicates significant difference with dose II. *-P<0.05, **-P<0.01.

Table 3: Effect of oral administration of AFENC on saluretic activity, natriuretic activity, diuretic index and diuretic activity

Treatment	Dose mg/kg	Saluretic activity	Natriuretic activity	Diuretic index	Diuretic activity
Group 1 Normal saline	25mg/kg	188.17±7.92	14.56±2.35		
Group 2 Furosemide	750mg/kg	208.54±7.62	18.87±2.09	1.28±1.54	
Group 3 AFENC	Dose 1 (200mg/kg)	210.38±6.85	20.79±3.45	1.28±0.34	1.01±0.059
Group 4 AFENC	Dose 2 (400mg/kg)	247.47±12.01^{a*b**c*}	22.69±1.64	1.80±0.39	1.50±0.037^{a**b**}

Values are expressed as mean ± SE for four animals in each group. One way Anova followed by Tukey test. a-indicates significant difference with normal control groups, b- indicates significant difference with diuretic control groups, c-indicates significant difference with dose I, d- indicates significant difference with dose II. *-P<0.05, **-P<0.01

Creatinine has been found to be a reliable indicator of kidney function and the serum creatinine level is an important diagnostic tool to assess renal functions. Serum creatinine and urine creatinine levels were found to be significantly decreased in the extract-treated group in a dose-dependent manner. Creatinine clearance test measures how well creatinine is removed from blood by kidneys and it gives a better insight on the functioning of kidneys than that obtained through the blood creatinine test. The results revealed that the AFENC extract can create a significant dose-dependent decrease in creatinine clearance levels. Glomerular filtration rate (GFR) is another important index in the clinical management of nephrourological problems. GFR is the measure of fluid filtered from the renal glomerular capillaries into the Bowman's capsule per unit time[14] . GFR rate increased in group 3and 4 in dose-dependent manner and animals receiving the dose at 400 mg/kg have high glomerular filtration rate. From the previous studies, it was reported that the increased GFR is due to the enhanced glomerular blood flow triggered by the drug[15] or due to decreased renal perfusion pressure in kidneys. The fruit extract may be endowed with such mechanism of action and this could be the reason for the increased GFR in the test drug.

Urinary excretion rate was found to be elevated in all the experimental groups compared to normal control animals. In this parameter also the AFENC at high dose had high urinary excretion rate, whereas low dose had the urinary excretion rate quantitatively similar to that of furosemide-treated ones **(Table 2)**. The saluretic and natriuretic activity of the fruit extracts exceeds the value of the standard diuretic drug and the dose 2 group also had high saluretic and natriuretic activity compared to the low dose (200 mg/kg) group. High diuretic activity was observed in animals receiving the dose of 400 mg/kg body weight **(Table 3)**. Group 3 (200 mg/kg of the extract) also possess diuretic activity and its value is quantitatively similar to that of furosemide. The diuretic activity of a drug is considered to be good, if the diuretic value is 1.50 and if the value is between 1.00 and 1.50 the drug has moderate diuretic activity. Diuretic activity of the drug is small if the value ranges from 0.72 to 1.00. If the value is below 0.72, the drug is having no diuretic activity[16]. In this respect the *N. cadamba* fruit extract at high dose has good diuretic activity (1.50±0.037) and at low dose has moderate diuretic activity (1.01±0.059). The diuretic property of the extract may be due to the synergistic action of [HCO_3^-/Cl^-], [HCO_3^+/H^+] exchangers and the [N^+/H^+] antiporter[15]. From the result of the present study it was evident that AFENC has diuretic activity in a dose-dependent manner in experimental animals.

Several studies have revealed that plants having antilithiatic activity also possess diuretic effect[17]. This results substantiates the curative properties of the aqueous fruit extract of *N. cadamba* against calcium oxalate induced urinary calculi. Diuretics have proved their role in the treatment of hypertension and enhancing the effect of other antihypertensive drugs. They generally relieve hypertension by increasing the excretion of sodium and water[18] and similar effects are observed in this study. So the test drug possesses anti-hypertensive action.

Among different types of diuretics, loop diuretics are the most powerful, and they reduce NaCl reabsorption in the thick ascending limb of the loop of Henle. This is achieved by inhibiting the Na-K-2Cl carrier in the luminal membrane in this segment, thereby minimizing the entry of luminal sodium into the cell[19]. Loop diuiretics also have direct effect on vasculature including increase in renal blood flow. But thiazide diuretics inhibit Na^+-Cl^- symport in the luminal membrane

of the epithelial cells thereby inhibiting NaCl reabsorption from the kidney[20]. Micropuncture experiments by Reger and Wangnam reported that high-ceiling diuretics enhance Na^+, Ca^+ and Cl^- excretion[21]. From the present study it was observed that Ca^+ excretion increases in the fruit extract-treated groups. All these results strengthen the loop diuretic property of *N. cadamba* fruit extract and the test drug (AFENC) has the mechanism of action similar to that of loop diuretics (furosemide).

Previous studies on diuretic activity of *Triumfetta rhomboidea*[22] and *Centella asiatica*[23] have confirmed the presence of phytochemicals such as steroids, flavanoids, saponins and organic acids[24, 25] and these have been found to be the main factor in the diuretic activity of plants. The preliminary phytochemical analysis of *N. cadamba* fruit revealed the presence of steroids, terpenoids, coumarins, tannins, saponins and carbohydrate. A polysaccharide composed of xylose, mannose and glucose in the ratio 1:3:5 was isolated from the seeds of *N. cadamba*. The presence of these phytochemicals could be the reason for the observed diuretic property of the fruits. The mentioned phytochemicals have different biological effects in our body. Antidiuretic hormone (ADH) plays an important role in urine volume[26]. Data of that study suggested that the test drug may affect the hypothalamopituitary axis and inhibit the ADH secretion or stimulate the release of natriuretic peptide, which promote the secretion of Na^+ and H_2O.

In conclusion the present study confirmed the effectiveness of the fruit extract as a diuretic with loop-diuretic like properties, thereby significantly increasing the urine volume, electrolyte concentrations, glomerular filtration rate, creatinine clearance, saluretic, natriuretic and diuretic activity in a dose-dependent manner. Being a natural remedy, the plant has been used as an effective diuretic against many clinical problems. Even though the tree is provided with numerous fruits, studies on the fruit are very few. This study thus provides the scientific evidence for the diuretic activity of fruit of *N. cadamba*. However, further studies are needed to isolate and characterize the active mechanism behind this diuretic activity.

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