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Research Article

A COMPARATIVE STUDY ON THE DIURETIC ACTIVITY OF *NEOLAMARCKIA CADAMBA*

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ABSTRACT

The present study aimed to evaluate the diuretic property of fruits of *Neolamarckia cadamba* in both aqueous and methanolic extract in wistar albino rats. The study was compared with the standard diuretic drug furosemide. Urine volume, pH, conductivity, UE rate, urine creatinine, serum creatinine, creatinine clearance, GFR, saluretic activity, natriuretic activity, diuretic activity, urinary calcium and phosphorus were investigated. Both extract showed a dose dependent increase in diuretic activity and aqueous extract was found to be more effective in increasing the urine volume. The extract showed the properties of both loop and thiazide diuretics. Hence, the study revealed the diuretic activity of fruits of *N. cadamba* and is comparable with the standard diuretic drug.

Keywords: diuretic activity, *Neolamarckia cadamba*, GFR, creatinine clearance

INTRODUCTION

Diuretics are the substances or drugs which act on kidney and promote diuresis (flow of urine) in various ways[1]. Diuretics generally work by increasing the urine expulsion rate and urinary sodium from the body thereby reduces the volume of blood[2]. Fluid retention disease conditions of renal, hepatic and cardiac system mainly relies on diuretic therapy which used in the treatment of volume overload in the body. These diuretic agents inhibit the renal ion transporters and are used in many life threatening disease conditions such as renal calculi, renal failure, hypertension, diabetes insipidus and conjunctive heart failure[3]. Major purpose of diuretic drug is to adjust the balance between water and electrolyte concentration in the body to promote the elimination of waste products and toxic substances from the body and also involved in the proper functioning of the kidney. In the field of medicine there are different categories of diuretics and diverse diuretic drugs for various purposes [4].

Around 20% of all men and women of older years and 50% of those over 80 years of age, use diuretic every day for many years. This illustration revealed that diuretic drugs rank high in the list of commonly prescribed drugs[5]. Many developed and developing countries widely practiced and accepted the use of ayurvedic system of medicine. WHO states that nearly 80% of global population depends on plant medicine in one way or other for the primary health care. The objectives of present experiment are to find out the diuretic activity of aqueous fruit extract of fruits of *N. cadamba* [6]with methanol fruit extract to investigate which solvent extract is effective in eliciting diuresis.

MATERIALS AND METHODS

Plant material

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

Preparation of 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. The dried fruits were milled in a mechanical grinder to make it powder. The aqueous fruit extract (AFNC) and methanol fruit extract of *N. cadamba* (MFNC) were prepared by keeping the powdered plant material in soxhlet extraction apparatus for 74 hrs, using distilled water and methanol as solvents respectively. After the period of extraction, the extract 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 animal

Healthy adult male albino rats of Wistar strain weighing 150–200 g were used for the diuretic activity. All 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)).

Assessment of diuretic activity

Pharmacological evaluation of diuretic activity

Adult healthy overnight fasted rats were randomly divided into six groups and each group consists of eight animals. Diuretic activity was assessed by the method of Lipschitz *et al.* (1943). Animals in all the groups received normal saline (25 ml/kg) orally using gastric intubation tube. Group I which served as the normal control received only saline. Animals which received the standard diuretic drug furosemide were taken as group II. Group III and group IV consisted of the aqueous fruit extract-treated

group at a dose of 200 mg/kg and 400 mg/kg body weight respectively. Group V and VI were supplemented with MFNC at a dose of 200 mg/kg and 400 mg/kg body weight. After the administration of the treatments, animals were kept in metabolic cages, specially designed to separate urine and feces. Animals were deprived off food and water during the urine collection period. Urine samples of all animals were collected in a measuring cylinder for a period of 24hr. After the treatment period, blood samples were collected from the tail vein and centrifuged to separate the serum.

Analytical procedures

Urine samples were analyzed for its volume, pH, conductivity, Na+, K+, Ca+ (O-cresolphthalein complexone method), PO_4 (UV - molybdate method) chloride and creatinine (Alkaline picrate method). Blood samples were used to determine serum creatinine (Alkaline picrate method) 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 determined using flame photometer (Systronics 129). Chloride content was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as indicator by Volhard's method[7]. The other parameters such as urinary excretion rate, diuretic index, diuretic, saluretic and natriuretic activities were monitored for individual rats and were calculated using standard formulae [8].

Statistical analysis

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

RESULTS

The present investigation is focused on the diuretic activity of fruit of *N. cadamba* in two different solvent extracts in aqueous (AFNC) and methanol (MFNC). AFNC at high dose showed high urine volume (9±1.07ml) than its low dose treated ones (6±0.73ml) and the value is significant ($p<0.01$) with control groups (3.33±0.23ml). Both aqueous fruit extract and methanol fruit extract showed a dose dependent increase in the volume of urine but high increase in urine volume was noticed from AFNC (400mg/kg b. wt.) and the value is in the range of standard diuretic drug, furosemide (Fig. 2). Urine pH has no significant variation among the groups. Aqueous extract at dose 2 exhibited high conductivity value (72.75±3.71) when compared to all other groups and is statistically significant ($p<0.01$) with saline treated control groups and furosemide treated groups (Table 1). Group III and IV of AFNC treated groups showed elevated sodium concentrations in a dose dependent manner. A significant increase ($p<0.01$) in urinary sodium was observed from group V (55.39±6.83) and group VI (64.23±5.68) (Table 4). Both AFNC and MFNC treated groups exhibited an elevated urinary sodium excretion than other groups (Fig. 3).

When compared to group II, group III and IV of AFNC increased the concentration of potassium to 3.79±0.92 and 4.65±0.31 respectively. Here the value is not statistically significant. But in the case of MFNC, a decrease in urinary potassium was observed when compared to other groups. In the case of AFNC treated groups, the observed value for dose 1 is 138.5±2.6 and for dose 2 is 141±1.29. No significant increase in chloride concentration was observed in AFNC treated groups. While in MFNC administered groups, dose 1 and dose 2 exhibited increase in chloride level when compared to control (Table 2). Both doses of AFNC and MFNC showed increased urinary chloride concentration and are statistically significant ($p<0.01$) with normal saline treated group of rats (Fig. 3). Dose 2 of AFNC increased the urinary excretion rate and the values are near to that of group II (Table 3). Both doses of AFNC and MFNC groups showed a dose dependent increase in urinary excretion rate (Fig. 3).

The dose 1 of AFNC increased urine creatinine concentration to 0.19±0.03 and of dose 2 was 0.17±0.05. Group V and VI treated with MFNC also enhanced urine creatinine level when compared with group I and II. Serum creatinine level is found to be increased in furosemide administered group of rats (2.85±0.29) while both fruit extract treated groups, the value is found to be decreased significantly. The results clearly showed that only high concentration (dose 2) of the AFNC can effectively reduce the serum creatinine concentration. This observation clearly demonstrated the effectiveness of the fruit extract (AFNC) in increasing creatinine clearance (Fig. 4). Group V and VI of MFNC administered rats showed a decreased creatinine clearance value of 0.22±0.05 and 0.17±0.01 respectively. The value of creatinine clearance of group V (0.22) was equivalent to that of control rats (0.23±0.05).

The analysis of glomerular filtration rate reported an increased glomerular filtration (GFR) in group IV treated with high dose (400mg/kg) of the fruit extract and is significant when compared to furosemide treated groups. Dose 1 of AFNC (group III) also showed elevated GFR. The results revealed that aqueous fruit extract increased the GFR when applied high dose. Saluretic activity was increased in group III (75.67±7.08) and group IV (111.12±6.005) at significant level ($p<0.01$) when compared to group I and II. Elevated saluretic activity was observed from dose 2 (400mg/kg) of AFNC compared to all other treatment groups. MFNC supplemented groups (group V and VI) exhibited saluretic activity higher than both normal and furosemide control rats (Table 4). Both dose 2 groups of AFNC and MFNC supplemented animals exhibited elevated saluretic activity than dose 1 (Fig. 5).

The natriuretic activity of dose 1 of AFNC (group III) is more or less same in the group II whereas in group IV supplemented with dose 2 of AFNC has a mean natriuretic activity of 22.90±1.64 which is statistically significant with normal saline loaded groups (group I). Like AFNC administered groups, in methanol fruit extract treated groups (group V and VI) also increased natriuretic activity. Among all the treatment groups, the group VI which received high dose of MFNC reported high natriuretic activity (23.27±1.86) (Fig. 5).

Among the treatment groups, rats treated with standard diuretic drug exhibited an elevated mean diuretic index value (3.06±1.54). A mean value of 1.82±0.34 is the reported diuretic index in group III (AFNC, dose 1). Both treatment doses of MFNC (group V and VI) reported diuretic index of 1.21±0.24 and 1.51±0.75 respectively. Highest diuretic index in fruit extract observed from group IV, treated with 400mg/kg of AFNC and the value is 2.72±0.30, which is found near to the diuretic index of furosemide treated group II (Table 4). When comparing the diuretic activity of different doses of fruit extract, high dose supplemented aqueous fruit extract (group IV) showed highest diuretic activity (0.88). The diuretic activity of AFNC at its low concentration (group II) is 0.59, which is found to be higher than both dose 1 and dose 2 of methanol fruit extract of *N. cadamba.* Dose dependent increase in calcium excretion was noticed in the aqueous fruit extract supplemented groups of animals. However, the data of urinary calcium decreased with increase in dose of MFNC (Table 5). A significant ($p<0.01$) decrease in urinary

calcium was noticed in dose 2 of MFNC (group VI) when compared to furosemide supplemented group (group II).

Like calcium, urinary phosphorus showed increased concentration in group II (12.71±0.95) which is higher than control group (7.81±1.48). Dose 1 and 2 of AFNC (group III and IV) exhibited an increase in concentration of urinary phosphorus with increase in dose of AFNC. When compared to group I and II, group V received 200mg/kg of MFNC (dose 1) reported phosphorus concentration of 2.48±0.04 and its high dose was observed with decreased phosphorus level in urine (1.05±0.17). The data clearly revealed that AFNC increased the urinary calcium and phosphorus levels with the increase of extract but MFNC decreased calcium and phosphorus concentration with increase in concentration of the extract (Table 5).

Table 1. Effect of AFNC and MFNC on urine volume, pH and conductivity

Treatment	Dose (mg/kg)	Urine volume (ml/24hr)	Urine pH	Urine conductivity
Group I Normal saline	25mg/kg	3.33±0.23	9.14±0.05	30±2.52
Group II Furosemide	750mg/kg	10.1±1.17	8.89±0.07	36.5±1.89a**
Group III AFNC	Dose1 (200mg/kg)	6.0±0.73	9.06±0.13	64.25±2.52 b*
Group IV AFNC	Dose 2 (400mg/kg)	9.0±1.07	9.24±0.05	72.75±3.71 b**
Group V MFNC	Dose1 (200mg/kg)	4 .0±0.24	8.98±0.15	47.5±3.571
Group VI MFNC	Dose 2 (400mg/kg)	5.0±0.38	9.95±0.06	50±2.096

Each value is the mean ± SEM for 8 animals, a-indicates significant difference with normal control groups, b indicates significant difference with diuretic control groups. *-P<0.05, **-P<0.01.

Table 2. Effect of AFNC and MFNC on urinary electrolyte excretion

Treatment	Dose (mg/kg)	Urinary excretion rate	Urine creatinine (mg/dl)	Serum creatinine (mg/dl)	Creatinine clearance (ml/min)	GFR (ml/min)
Group I Normal saline	25mg/kg	55±5.57	0.04±0.01	0.58±0.054	0.23±0.053	0.19±0.03
Group II Furosemide	750mg/kg	168.33±5.91	0.03±0.01	2.85±0.29 a**	0.11±0.016	0.09±0.01
Group III AFNC	Dose 1 (200mg/kg)	100±5.91	0.19±0.03 a**b**	1.53±0.16 a**b**	0.75±0.09	0.61±0.07
Group IV AFNC	Dose 2 (400mg/kg)	150±6.43	0.17±0.05 a*b*	0.69±0.08 b**	2.21±0.05 a**b**	1.79±0.06 a*b*
Group V MFNC	Dose 1 (200mg/kg)	66.66±4.80	0.10±0.03 a*b*	1.86±0.01	0.22±0.05	0.18±0.04
Group VI MFNC	Dose 2 (400mg/kg)	83.33±7.76 a*	0.08±0.01	2.41±0.10	0.17±0.01	0.14±0.01

Each value is the mean ± SEM for 8 animals, a-indicates significant difference with normal control groups, b indicates significant difference with diuretic control groups. *-P<0.05, **-P<0.01.

Table 3. Effect of AFNC and MFNC on urinary parameters

Treatment	Dose (mg/kg)	Urinary electrolyte excretion		
		(Na+) (mEq/l)	(K+) (mEq/l)	(Cl-) (mEq/l)
Group I Normal saline	25mg/kg	38.58±6.60	4.12±0.73	97±1.63
Group II Furosemide	750mg/kg	43.17±5.17	2.41±0.46	145±3.00
Group III AFNC	Dose1 (200mg/kg)	71.88±9.27	3.79±0.92	138.5±2.63a**
Group IV AFNC	Dose 2 (400mg/kg)	106.47±7.3 a**b**	4.65±0.31	141±1.29 a**
Group V MFNC	Dose1 (200mg/kg)	55.39±6.83	3.66±0.42	142.5±4.7 a**
Group VI MFNC	Dose 2 (400mg/kg)	64.23±5.68	2.76±0.37	153.5±1.25 a**

Each value is the mean ± SEM for 8 animals, a-indicates significant difference with normal control groups, b indicates significant difference with diuretic control groups. *-P<0.05, **-P<0.01.

Table 4. Effect of AFNC and MFNC on diuretic activity

Treatment	Dose (mg/kg)	Saluretic activity	Natriuretic activity	Diuretic index	Diuretic activity
Group I Normal saline	25mg/kg	42.7±7.018	9.36±2.35		
Group II Furosemide	20mg/kg	45.58±5.551	17.91±2.09	3.06±1.54	
Group III AFNC	Dose 1 (200mg/kg)	75.67±7.087	18.97±3.45	1.82±0.34	0.59
Group IV AFNC	Dose 2 (400mg/kg)	111.12±6.005 a*b**	22.90±1.64	2.72±0.39b*	0.88
Group V MFNC	Dose 1 (200mg/kg)	59.05±7.087	15.13±1.847	1.21±0.24	0.40
Group VI MFNC	Dose 2 (400mg/kg)	66.99±6.005	23.27±1.864 a*b*	1.51±0.75	0.49

Each value is the mean ± SEM for 8 animals, a-indicates significant difference with normal control groups, b indicates significant difference with diuretic control groups. *-P<0.05, **-P<0.01.

Table 5. Effect of AFNC and MFNC on urinary calcium and phosphorus

Treatments groups	Dose (mg/kg)	Calcium (mg/24hr)	Phosphorus (mg/24hr)
Group I Normal saline	25ml/kg	1.16±0.19	7.81±1.48
Group II Furosemide	20mg/kg	2.29±0.82	12.71±0.95
Group III AFNC	Dose 1 (200mg/kg)	0.78±0.12	7.31±1.05
Group IV AFNC	Dose 2 (400mg/kg)	1.42±0.61	14.87±2.23
Group V MFNC	Dose 1 (200mg/kg)	1.71±0.36	2.48±0.40a**
Group VI MFNC	Dose 2 (400mg/kg)	0.46±0.16 b**	1.05±0.17a**b**

Each value is the mean ± SEM for 8 animals, a-indicates significant difference with normal control groups, b indicates significant difference with diuretic control groups. *-P<0.05, **-P<0.01.

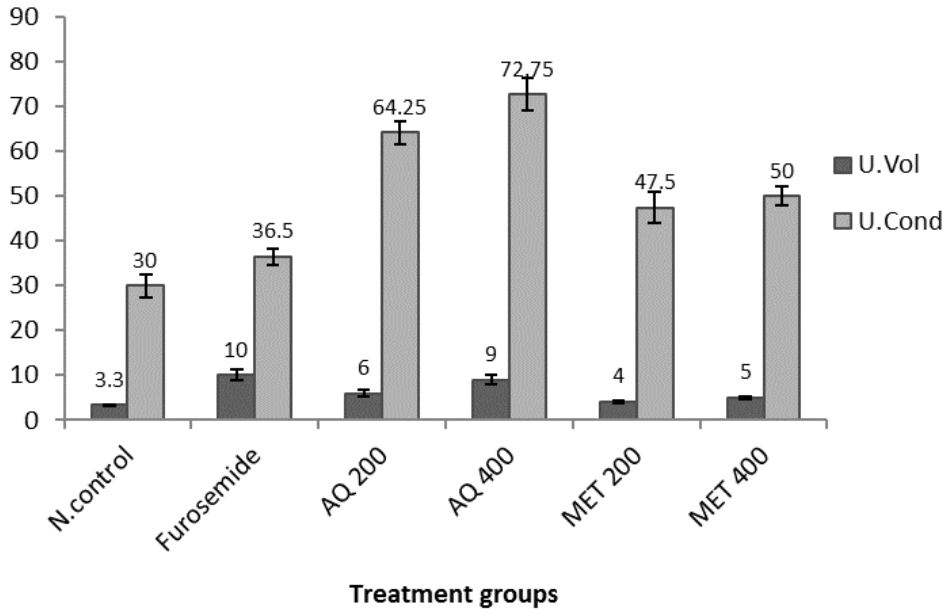

Fig. 2. Effect of AFNC and MFNC on urine volume and urine conductivity

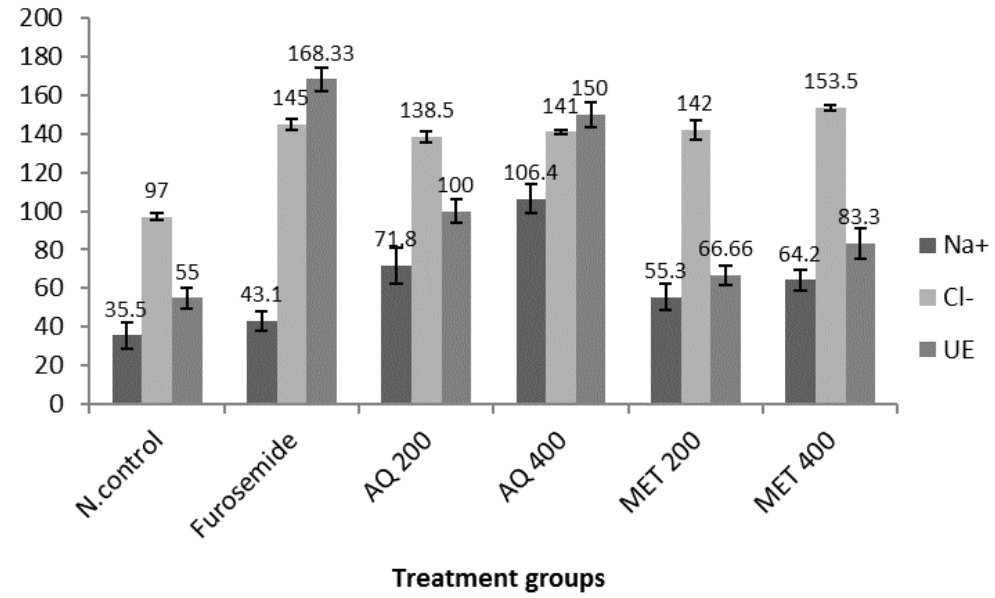

Fig. 3. Effect of AFNC and MFNC on urine electrolyte concentration and urinary excretion rate

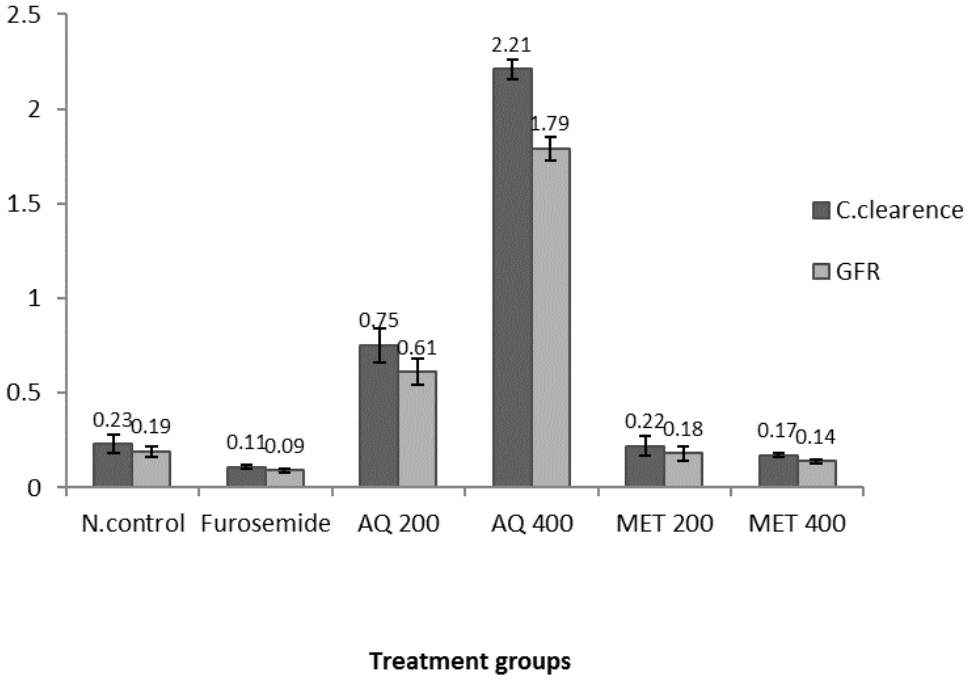

Fig. 4. Effect of AFNC and MFNC on creatinine clearance and glomerular filtration rate

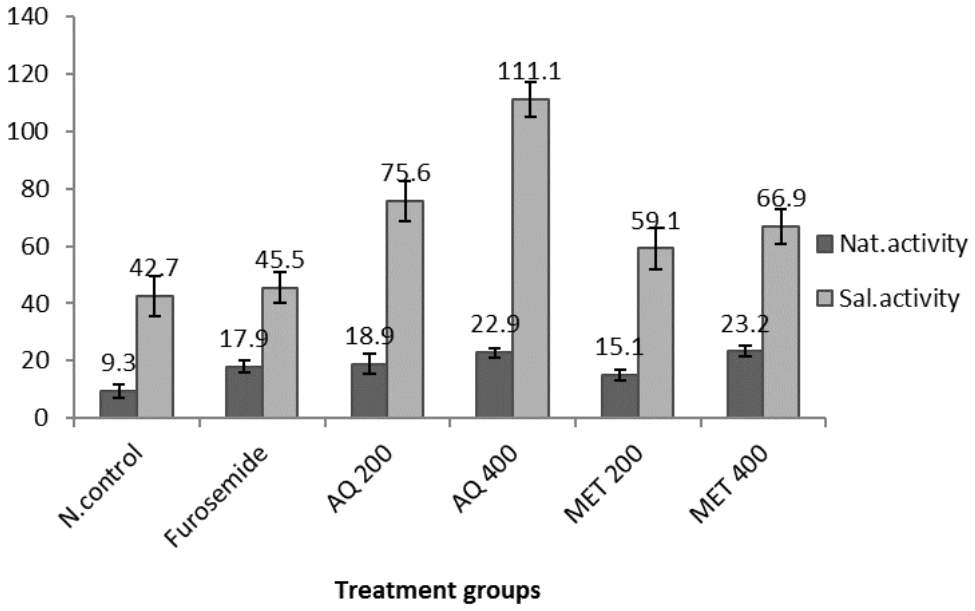

Fig. 5. Effect of AFNC and MFNC on natriuretic and saluretic activity

DISCUSSION

The present study was carried out to determine the diuretic activity of AFNC and MFNC in Wistar albino rats. The diuretic activity of furosemide (LASIX) was taken as standard diuretic for comparing the pharmacological responses[9]. An ideal diuretic is beneficial in the treatment of edema, congestive heart failure, chronic renal failure and nephritic syndrome is yet to be discovered[10].

The results revealed that the fruit extract of *N. cadamba* increased the volume of urine output. From this it is evident that the fruit extract has the capability to increase the volume of urine which is one of the most important criteria for a diuretic substance. The ethano pharmacological studies indicated that secondary compounds of plants increase urine output. The urine conductivity is used as an indirect measure of ionic content of urine[11] and is increased in both aqueous and methanol fruit extract treated groups when compared to group I and II. The increased conductivity value and urine volume suggested that the diuretic action of the fruit extract is ascribed to the saluretic activity rather than aquaretic action which is unlikely to be operating in the present study.

The aqueous fruit extract increased the electrolyte concentration of sodium and chloride significantly ($p<0.01$). The diuretic activity of the extract is indicated by increased water and sodium ion excretion[12]. The marked increase in urinary sodium level in fruit extract supplemented groups suggests that the fruit extract inhibit the reabsorption of sodium in the nephrons of kidney[13]. The urinary excretion of chloride ions not elevated significantly in the aqueous fruit extract when compared with MFNC and the results indicated that the AFNC has potent natriuretic action than MFNC[14]. But AFNC increased urinary potassium level with increase in dose of the extract and the value is not statistically significant. All these observations suggested that AFNC likely to posses the diuretic action of high ceiling diuretic, the loop diuretic.

Among different types of diuretics, loop diuretics are the most powerful. They block Na^+-K^+-$2Cl^-$ symport in the loop of Henle and prevent the reabsorption of NaCl and KCl in the thick ascending limb of loop of Henle[15]. 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[16]. In the present study, AFNC elevated the excretion of calcium with increasing dose of the aqueous fruit extract. All these results strengthen the loop diuretic property of *N. cadamba* fruit extract and the test drug (AFNC) has the mechanism of action similar to that of loop diuretics (furosemide).

MFNC elevated the concentration of urinary sodium and chloride with the increase in dose of the methanol fruit extract. Unlike AFNC, excretion of potassium and calcium in urine was found to be decreased as the dose of extract increases when compared with the saline treated group of rats. The thiazide diuretics inhibit Na^+-Cl^- symport in the luminal membrane of the epithelial cells thereby inhibiting NaCl reabsorption from the kidney[17] and enhance the reabsorption of calcium by inhibiting Na^+- Cl^- symport in the luminal membrane of epithelial cells and also enhancing the activity of Na^+- Ca^{++} exchanger in the basolateral membrane of epithelial cells[18]. The results revealed that MFNC have the action of thiazide like diuretics. Decreased potassium excretion in MFNC administered group could be attributed to the potassium conserving action of the test drug. MFNC elevated the concentration of Na^+, K^+, Na^+/K^- ratio and this observation suggests that the fruit extract can also act as osmotic diuretic[19]. Creatinine is a reliable indicator of kidney function and the serum creatinine level is an important diagnostic tool and as an index to assess renal functions[20]. 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 AFNC extract created a significant dose-dependent increase in creatinine clearance level.

GFR rate increased in group III and IV in a dose-dependent manner and animals received the dose of 400 mg/kg have high GFR. MFNC treated groups also showed increased GFR. From

the previous studies, it was reported that the increased GFR is produced by the enhanced glomerular blood flow triggered by the administered drug[21]. The increased GFR may attribute to the action of the test drug with structural components of the glomerular membrane or its direct effect on arteriole wall to induce glomerular blood flow[22].

The saluretic and natriuretic activities of the fruit extract exceeds the values of furosemide treated groups and the dose 2 of AFNC also had high saluretic and natriuretic activities compared to the low dose (200 mg/kg). The high dose of MFNC (group VI) also exhibited an increased saluretic and natriuretic activity. The diuretic property of the extract could be due to the synergistic action of [HCO_3^-/Cl^-], [HCO_3^+/H^+] exchangers and the [Na^+/H^+] antiporter as described by others[21]. The increasing ratio of Na^+ to K^+ in excreted urine also revealed the potential nature of the fruit extract in increasing Na^+ excretion than K^+ which is a major characteristic of a good diuretic drug[23, 24].

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