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RESEARCH ARTICLE

Effects of Methanolic Extract of *Oxystelma esculentum* on Diuresis and Urinary Electrolytes Excretion in Rats

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ABSTRACT

The diuretic activity of methanol extract of *Oxystelma esculentum* aerial parts (MEOE) was studied in male Wister albino rats at 5h and 24h intervals. The animals were divided into 5 groups: control, urea, furosemide, 200mg/kg and 400mg/kg MEOE. The MEOE was administered intraperitoneally (i.p) and all animals were pretreated with saline before commencing the experiment. The urine volume (in mL) and electrolytes excretion (Na^+, K^+, Ca^{2+} and Cl^-) at 5h and 24h duration were measured. The urine output increased significantly in urea, furosemide and both MEOE groups (p<0.001). MEOE increased the urine volume and electrolytes balance in a dose dependent manner. The results indicate that MEOE is an effective hypernatramic, hyperkalaemic, hypercalcemic and hyperchloremic diuretic, which supports the traditional claim about the *Oxystelma esculentum* being used as a diuretic.

Keywords: *Diuretic activity, Methanol extract, Oxystelma esculentum, Urine volume, Electrolytes excretion.*

Plant medicine was commonly used for traditional treatment of some renal diseases and a lot of plants were reported to show significant diuretic activity [1]. Many investigators demonstrated that studies of herbal plant used in traditional medicine as diuretics, were in progressive elevation in the last decades [2], and might be a precious tool used in human disease treatment.

Oxystelma esculentum R. Br. (Asclepiadaceae) is a perennial twining herb. It is distributed throughout the plains, on hedges and among bushes usually near water and lower hills of India, Ceylon and Java [3,4]. The decoction of the plant is used as gargle in aphthous ulcerations of mouth and in sore throat. The root is considered specific for jaundice and the milk sap is used as a wash for ulcers [5-7]. In Ayurveda, the plant is a diuretic, aphrodisiac, anthelmintic and anti-bronchitis, is useful in leucoderma and the fruit is expectorant, anthelmintic. The fruit juice is used in gonorrhoea and pain in muscles [8]. A cardenolide tetraglycoside, oxyline isolated from roots and polyhydroxy pregnane glycosides, alpinoside A, B and C from aerial parts of the plant were reported [9,10]

Drug induced diuresis are beneficial in many life threatening disease conditions such as congestive heart failure, nephritis, hypertension and pregnancy toxemia [11]. Urine/electrolyte excretion is regulated by the $[HCO_3^-/Cl^-]$; $[HCO_3^+ /H^+]$ and the $[Na^+/H^+]$ exchanges. These are major intracellular/extra cellular pH regulators mediated by carbonic anhydrase, carbonic hydrogenase and phosphorylase [12]. The purpose of this study was to evaluate the diuretic activity and its possible mechanism of action in the methanol extract from *O.esculentum* in animal models.

MATERIALS AND METHODS

Collection of Plant Material

The aerial parts of *Oxystelma esculentum* R. Br. used in this study were collected in Srirangapatnam, Near Mysore, Karnataka. They were identified by H.O.D, Department of Botany, Kuvempu First Grade College, Channapatna, Karnataka, India. A voucher specimen No DAKJU-02/2005 has been deposited in our laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India for future reference.

Preparation of Extract

The collected aerial parts were air dried, pulverized in to a coarse powder and sieved. The dried powdered material was defatted with petroleum ether ($60\text{-}80^0$) and

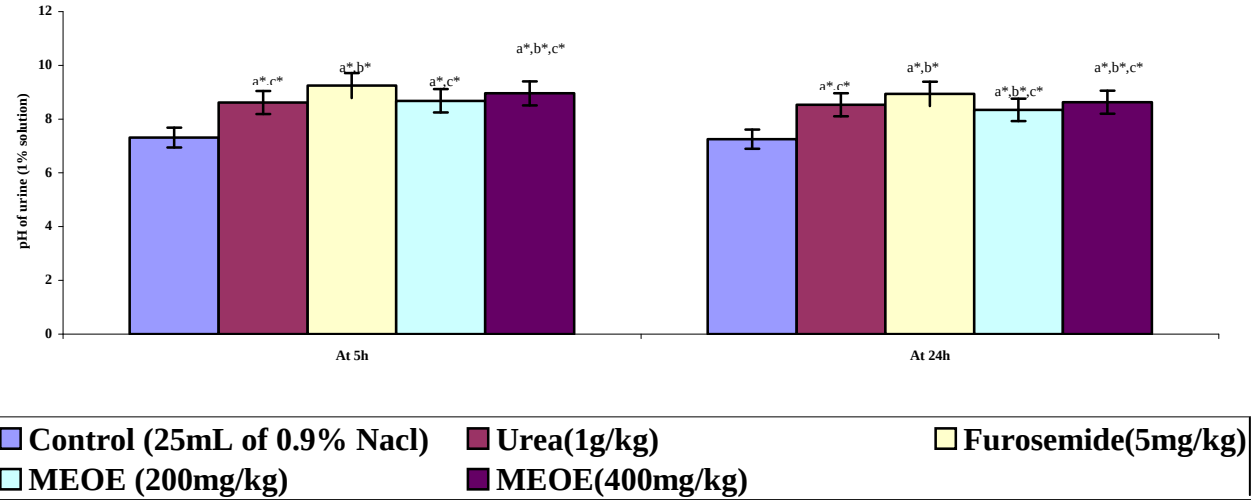

Fig 1. Effect of methanol extract of *Oxystelma esculentum* (MEOE) on pH in normal rats at 5th and 24th hour by intraperitoneal administration.
Values are expressed as mean ± SEM (Number of animals, n=6); a and a* indicates $p<0.05$ and $p<0.001$vs. Control; b and b* indicates $p<0.05$ and $p<0.001$vs. Urea; c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide.

was further extracted with methanol in a soxhlet apparatus. The extract was filtered and the solvent was removed by distillation under vacuum. The chemical constituents of the extract were identified by qualitative analysis [13]. The dried MEOE was suspended in distilled water and used for further studies.

Experimental Animals

Male Wister albino rats weighing 150-180g body weights were obtained from Indian Institute of Chemical Biology, Kolkata. All animals were maintained under environmentally controlled conditions of 24 ± 1^0C and 12 h light –12h dark cycle. The animals had free access to water and food and were acclimatized to laboratory condition at least 1 week before starting the experiments. The experiment was performed under standard conditions of temperature, light, humidity and noise.

Chemicals

Petroleum ether was obtained from Merck Limited, Mumbai; Methanol and urea were from Sisco Research Laboratories Pvt Ltd, Mumbai, Furosemide (Lasix) was obtained from Aventis pharma limited, Thane. All other chemicals used were of reagent grade.

Pharmacological Evaluation

Experimental design

Five groups of six male Wister albino rats each weighing between 150-180g/kg, b.w were used. All the animals received normal saline (25mL/kg, b.w) orally, prior to start of the experiment. Group I which received normal saline was treated as control. Group II received urea (1kg/kg). Group III received furosemide (5mg/kg). Group IV and V received the methanol extract at the dose of 200mg/kg and 400mg/kg body weight respectively. Immediately after administration of the drug, the rats were each placed in metabolic cages, specially designed to separate urine and faecal matter and observed at room temperature. The animals were denied food and water during the experiment. The urine volume was collected after 5h and 24h of the intraperitoneal administration [14,15]. The urine volume (mL/day) was measured and then assayed for Na^+, K^+, Ca^{2+} and Cl^- concentrations [16-18]. Na^+, $K^+$and Ca^{2+} were measured by aflame photometric method (Chemito 1020) while Cl^- was measured by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as an indicator [19]. The pH was determined using a pH meter (Mettler Toledo, Seven Easy) [20]. The instrument was calibrated with standard solutions containing different concentrations of sodium, potassium and calcium [21].

Diuretic Activity and Urinary Volume Excretion

The volume of the urine excreted after 5h and 24h of study by control, urea, furosemide and MEOE (200mg and 400mg/kg, b.w) was expressed as percent of the liquid administered giving rise to a measure of "Urinary excretion" (U.E)-independent of group weight [22], thus

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100$$

The ratio of (U.E) in test group and control group was denoted. Diuretic action, which was used as the measure of degree of diuresis [23].

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test group}}{\text{Urinary excretion in control group}} \times 100$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of drug}}{\text{Diuretic action of urea}} \times 100$$

$$= \frac{D}{U}$$

Table 1. Dose response diuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 5th and 24 th hour by intraperitoneal administration.

Groups	At 5h After Drug Administration				At 24h After Drug Administration			
	Urine volume (mL)	Urinary Excretion $(V_0/V_1)X100$	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)	Urine volume (mL)	Urinary Excretion $(V_0/V_1)X100$	Diuretic Action (UE_t/UE_c)	Diuretic Activity (DA_t/DA_u)
Control (25mL of 0.9% Nacl)	0.66±0.01	18.11	_	_	2.24±0.01	60.27	_	_
Urea(1g/kg)	0.83±0.02^{a*,c*}	21.56	1.19	_	2.50±0.01^{a*,c*}	64.94	1.08	_
Furosemide (5mg/kg)	2.13±0.01^{a*,b*}	54.34	3.00	2.52	4.33±0.01^{a*,b*}	110.46	1.83	1.69
MEOE (200mg/kg)	1.17±0.01^{a*,b*,c*}	32.50	1.79	1.50	2.90±0.01^{a*,b*,c*}	80.56	1.34	1.24
MEOE (400mg/kg)	2.17±0.01^{a*,b*}	55.64	3.07	2.58	4.40-±0.01^{a*,b*,c}	112.82	1.87	1.73

Values are expressed as mean ± SEM (Number of animals, n=6); V_0= Total urinary output; V_1= Total fluid input; UE_t = Urinary excretion in test group; UE_c=Urinary excretion in control group; DA_t= Diuretic action of the test sample; DA_u=Diuretic action of the Urea; a and a* indicates $p<0.05$ and $p<0.001$vs. Control; b and b* indicates $p<0.05$ and $p<0.001$vs. Urea; c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

Table 2. Electrolytes excretion (mMol/L), saliuretic and natriuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 5th hour by intraperitoneal administration.

Groups	Electrolytes excretion in mMol/L				$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25mL of 0.9% Nacl/kg)	133.43±0.04	55.51±1.15	59.66±0.34	105.33±0.66	238.76±0.70	2.40±0.05	0.5575±0.001
Urea (1g/kg)	150.26±3.08^{a,c*}	67.50±2.82^{a,c*}	68.79±0.54^{a,c}	146.66±1.76^{a*,c}	296.93±4.84^{a*,c*}	2.23±0.05^{a,c}	0.6740±0.010^{a*,c}
Furosemide (5mg/kg)	216.76±3.78^{a*,b*}	89.98±0.93^{a*,b*}	78.12±3.24^{a*,b}	176.33±3.17^{a*,,b}	393.09±6.95^{a*,b*}	2.40±0.01^{b}	0.5748±0.001^{b*}
MEOE (200mg/kg)	217.58±1.03^{a*,b*}	90.99±1.35^{a*,b*}	81.97±0.98^{a*,b*}	169.66±1.45^{a*,b}	387.24±2.49^{a*,b*}	2.39±0.02^{b}	0.5498±0.001^{b*,c}
MEOE (400mg/kg)	227.98±1.89^{a*,b8}	97.86±1.63^{a*,b*}	97.96±0.99^{a*,b*}	190.33±0.88^{a*,b}	418.31±2.77^{a*,b*}	2.33±0.02	0.5842±0.003^{a,b*}

Values are expressed as mean ± SEM (Number of animals, n=6) a and a* indicates $p<0.05$ and $p<0.001$vs. Control, b and b* indicates $p<0.05$ and $p<0.001$vs. Urea c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

Saliuretic, Natriuretic and Carbonic Anhydrase Inhibition

The sum of $Na^+$and Cl^- excretion was calculated as a parameter of saliuretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl^-/Na^+ + K^+$(ion quotient) was calculated to estimate carbonic anhydrase inhibition [24].

Statistical Analysis

Results are mean ± S.E.M. Statistical analysis of control and test data was determined by ANOVA (SPSS computer software). Simple one-way analyses of variance were used for different doses with in a group. A probability value of $p<0.001$ and $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical Analysis

The powdered materials were green in colour. The petroleum extract was dark green and had a musty odor while the methanol extract was blackish green and had a smelling flavor. The % yields were 5.22 and 14.60 for the petroleum ether and methanol extract respectively. The preliminary phytochemical screening of powdered *O.esculentum* revealed the presence of Glycosides, carbohydrates, flavonoids, phenolic compounds (Tannins), triterpenoids, saponins and steroids.

Effects on Urine Output and Diuretic Activity

The total urine volume over the period of 5h and 24 h were measured for the extracts, (200mg and 400mg/kg, b.w), standard diuretics (urea and furosemide) and control. Urea, furosemide and 200mg and 400mg/kg of MEOE increased the urine flow significantly at 5th h ($p<0.001$) and 24th h ($p<0.001$) when compared with control. The high dose excreted more than two fold the volume of urine as compared to control. MEOE increased urine flow in a dose dependent manner.

From the result it appears that MEOE exhibited diuretic activity at both doses (200mg and 400mg/kg, b.w) like furosemide at 5th h and 24th h and its effect was dose dependent (Table 1). The diuretic activity of a drug is considered to be good if it is above 1.50, moderate if it is within 1.00-1.50, little if it is between 0.72-1.00 and nil if it is less than 0.72. In this respect after the drug administration, MEOE showed good diuretic activity.

Effects on Electrolyte Excretion

The diuretic responses with its electrolyte excretion potency of the extract were highly moderate in comparison with control animals. The extract at doses of 200mg

Table 3. Electrolytes excretion (mMol/L), saliuretic and natriuretic activity of methanol extract of *Oxystelma esculentum* (MEOE) in normal rats at 24th hour by intraperitoneal administration

Groups	Electrolytes excretion in mMol/L				$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Ca^{++}	Cl^-			
Control (25mL of 0.9% Nacl/kg)	146.57±0.50	88.58±1.20	96.71±1.02	188.00±1.15	334.57±1.65	1.65±0.01	0.7995±0.001
Urea (1g/kg)	168.22±1.31^{a*}	101.79±1.64^{a*,c}	123.93±0.83^{a*,c}	218.00±1.73^{a*,c*}	386.22±3.04^{a*,c*}	1.65±0.03^{a,c}	0.8074±0.007^{c*}
Furosemide (5mg/kg)	170.44±1.21^{a*}	110.51±1.28^{a*,b}	140.47±1.02^{a*,b*}	242.00±1.15^{a*,b*}	412.44±2.37^{a*,b*}	1.54±0.007^{a,b}	0.8614±0.003^{a*,b*}
MEOE (200mg/kg)	171.77±0.86^{a*}	113.51±1.69^{a*,b*}	142.12±1.00^{a*,b*}	244.00±1.15^{a*,b*}	415.77±2.00^{a*,b*}	1.51±0.02^{a,b}	0.8553±0.006^{a*,b*}
MEOE (400mg/kg)	186.52±1.21^{a*,b*}	116.58±1.27^{a*,b*}	150.37±1.09^{a*,b*}	262.00±1.73^{a*,b*}	448.52±2.56^{a*,b*}	1.60±0.007	0.8645±0.006^{a*,b*}

Values are expressed as mean ± SEM (Number of animals, n=6) a and a* indicates $p<0.05$ and $p<0.001$vs. Control, b and b* indicates $p<0.05$ and $p<0.001$vs. Urea c and c* indicates $p<0.05$ and $p<0.001$vs. Furosemide

and 400mg/kg showed increase in Na^+, Ca^{2+} and Cl^- excretion, accompanied by the excretion of K^+. The highest dose (400mg/kg) enhanced significantly the urine excretion of sodium ($p< 0.001$), potassium ($p< 0.001$), calcium ($p< 0.001$) and chloride ($p<0.001$) compared with that of control.

Effects on Urine pH

The urine pH after administration of MEOE, 200mg and 400mg/kg body weight were 8.68±0.03, 8.96±0.008 and 8.34±001, 8.63±0.01 at 5th h and 24th h. Urea increased the urine pH 8.62 ±0.01and 8.54±0.0 compared to control. Similarly, after furosemide treatment the urine pH was 9.25±0.02 and 8.94±0.02 at 5th h and 24th h respectively, thus making the urine more alkaline. All the values were compared with that of control, 7.31±0.02 and 7.25±0.01 at 5th h and 24th h (Fig 1).

Effects on Natriuretic, Saliuretic and Carbonic Anhydrase Inhibition

From the Table 2 and 3, the methanol extract of *Oxystelma esculentum* at both doses (200mg and 400mg/kg) showed natriuretic and potent saliuretic activity comparable to that of control. No carbonic anhydrase inhibition was detected [24,25].

DISCUSSION

This study shows that MEOE produced striking increase in total urine output over a period of 5h and 24h. It also increased the excretion of sodium, calcium, chloride accompanied with potassium significantly. Therefore MEOE has been shown to possess significant diuretic, natriuretic and kaliuretic effects, which may be one of the reasons of its therapeutic application in various ailments such as treatment of renal disorders, treatment of liver disorders, ulcers and pain in muscles. Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria and cirrhosis of liver [26,27].

The diuretic activities of the extracts were highly potent when compared to control. However, there were significant differences in urinary excretion followed by diuretic action and diuretic activity. The extract caused increase urine elimination and increase in Na^+, K^+, Ca^{2+} and Cl^- excretion as compared to control (normal saline). The extract may possibly act by the synergistic action mechanism of the [HCO_3^-/Cl^-], [HCO_3^+/H^+] and the [Na^+/H^+] antiporter, to cause diuresis [28].

Furosemide acts by inhibiting electrolyte reabsortption in the thick, ascending limp of the loop of Henle [29]. Greger and Wangermann (1987) also found from micropuncture experiment that high ceiling diuretics enhanced Na^+, Ca^{2+} and Cl^- excretion; and microperfusion experiments revealed that there was a complete inhibition *in vitro* at luminal concentration of drugs in the range expected to occur *in vitro* [30]. The high ceiling diuretics may not affect K^+ loss [31-33].

At the 5th h and 24th h, the MEOE extracts showed change in urine output at both dose levels tested (200mg and 400mg/kg). The diuretic effect of the methanol extracts was significant at 5h and 24h. However, there is a slightly delayed effect at 24h. Even though, the diuretic activity at 24th h at both doses was significant. It showed the extracts acted in time and dose dependent manner which could have been as a result of absorption of the active principle(s) in the crude preparations or the extracts could have been stimulating *in vivo* a diuretic compound(s). Both doses of the methanol extract induced a significant increase in urine, Na^+, Ca^{2+} and Cl^-, accompanied by a significant excretion of K^+. This is a characteristic of high ceiling diuretic. No carbonic anhydrase inhibition was detected.

The 5h and 24h cumulative urine output induced by the extracts and standard drugs were statistically significant compared with control (saline treated). The high dose produced the highest urine volume and electrolyte output over the 5h and 24h period, and the low dose produced significant urine output and electrolyte excretion but it was less compare with furosemide and more or less similar to that of urea. It was reported by Nilve-

ses et al (1989) that an increment of the urine output in rats might result from high potassium content in the plant extract [34]. The pH values were also alkaline as compare with control.

The data reported in the present work indicate that the MEOE showed good diuretic activity, in comparison with furosemide high ceiling diuretic agent [35]. It can be observed that the methanol extract of *Oxystelma esculentum* is an effective hypernatramic, hyperchloremic, hypercalcemic and hyperkalaemic diuretic; which correlate well with the traditional use of the plant as a diuretic. From the observations showed, MEOE had similar diuretic spectrum to that of furosemide. Therefore, further researches are ongoing to find out the active principles responsible for the activities in our laboratory.

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