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PII: S0378-8741(09)00227-X
DOI: doi:10.1016/j.jep.2009.04.014
Reference: JEP 5512

To appear in: *Journal of Ethnopharmacology*

Received date: 11-5-2007
Revised date: 10-12-2008
Accepted date: 6-4-2009

Please cite this article as: Adam, Y., Somchit, M.N., Sulaiman, M.R., Nasaruddin, A.A., Zuraini, A., Bustamam, A.A., Zakaria, Z.A., Diuretic Properties of *Orthosiphon stamineus Benth*, *Journal of Ethnopharmacology* (2008), doi:10.1016/j.jep.2009.04.014

Revised Manuscript Ms No. JEP-D-07-00778
Ethnopharmacological Communications

Diuretic Properties of *Orthosiphon stamineus Benth*

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Abstract

Ethnopharmacological relevance: Orthosiphon stamineus has been used in traditional medicine for centuries especially to treat diseases of the urinary system.

Aim of the study: To investigate the diuretic activity, to elucidate its possible mechanism and evaluate the renal effects of *Orthosiphon stamineus* extract.

Materials and methods: Water extracts were administered orally at doses of 5 and 10 mg/kg to Spraque-Dawley rats and the control groups were given commercial diuretic drugs either furosemide or hydrochlorthiazide at 10 mg/kg. Urine volume, urine pH, urine density and urine electrolytes were determined every hour for 4 hours. Blood was assayed for glucose, albumin, blood urea nitrogen (BUN) and creatinine.

Results: O. stamineus extract exhibited dose-dependent diuretic activity. However, excretion of Na^+ and Cl^- was not markedly elevated, but urinary excretion of K^+ was significantly increased. *O. stamineus* extracts slightly increased the serum BUN, creatinine and blood glucose level. Although, these levels were statistically significantly when compared to control but these levels were still within normal range.

Conclusion: O. stamineus exhibited diuretic activity, but was less potent than furosemide and hydrochlorothiazide. Care should be taken when consuming this herb as slight increase of kidney function enzymes was recorded.

Keywords: Diuretic, Electrolytes, *Ortosiphon stamineus*

1. Plant.

Orthosiphon stamineus or Cat's Whiskers or "misai kucing" in Malay from the family Lamiaceae is a native plant of South East Asia. *O. stamineus* is a perennial herb, 0.3-1 m high, having stem 4-angled. The leaves are simple, opposite, ovate-oblong-lanceolate, elliptic or rhamboid, 2-4 cm wide, 4-7 cm long, flowers white or pale lilac, stamens exceeded more than 2 cm from the corolla-tube (Wiart, 2000).

The plants were collected from the Sepang District of Selangor, Malaysia and were verified. Samples were deposited in the Phytomedicinal Herbarium Institute of Bioscience, Universiti Putra Malaysia (Voucher specimen No. SK 315/06).

2. Uses in traditional medicine.

This plant has been used in traditional medicine for centuries to improve general health, treatment of kidney diseases, bladder inflammation, gout and diabetes (Wagner, 1982; Akowuah et al., 2005). *O. Stamineus* also being used to treat rheumatism, tonsillitis and menstrual disorder (Awale et al., 2003).

3. Previously isolated classes of constituents

Phenolic compounds such as lipophilic flavones, caffeic acid derivatives (rosmarinic acid and 2,3-dicaffeoyltartaric acid) (Sumaryono et al., 1991; Akowuah et al., 2004), sinensetin and methoxy flavones (Pietta et al., 1991), diterpenes, betulinic acid, oleanolic acid and sitosterol (Tezuka et al., 2000) have been isolated from *O. stamineus*.

4. Materials and Methods

4.1 Preparation of the extracts

The plants were prepared using the method described previously by Somchit et al., (2003) with slight modification. Briefly, the freshly collected leaves were washed and weighed. After that the parts were cut in small and dried at 50 to 60^{o}C for 5 days. The dried leaves were weighed and then grounded to powder form. The powder form of plants were weighed and kept in an airtight plastic bag at room temperature. The dried powder was soaked in 2 liter of distilled water for 2 hours at room temperature. Then, the mixture was boiled until the volume decreased to one-third of the initial volume. After boiling, the mixture was cooled and filtered using Whatman filter paper No.1. The filtrate was then freezing dried and final extract was stored at minus 20^{o}C prior to use (yield: *O. stamineus* 11% w/w). The extracts were reconstituted with distilled water prior to diuretic assessment in different concentration of the treatment.

4.2 Experimental Animals

Adult male Spraque-Dawley rats (180-200 g) were housed in cages of three, and acclimatized to the laboratory environment for a minimum one-week prior to experiment. They were maintained under standard and uniform laboratory condition with free access to rat pellet diet and water *ad libitium*. All procedures were approved by the institutional Ethical committee.

4.3 Diuretic assessment and treatment groups

Methods Sripanidkulchai et al., (2001) were used for the determination of diuresis. Extracts and drugs were administered orally. Animals were given bicarbonate saline solution (40 ml/kg) 30 minutes before the experiment. They were divided into 5 groups (n=4/group) and placed in an individual metabolic cage. Negative control (Group 1) was given 1.0 ml/rat of distilled water orally. The positive control groups (Group 2 and 3) were given furosemide and hydrochlorothiazide (Sigma Chemicals, US) at 10 mg/kg in distilled water respectively. Treatment groups (Groups 4 and 5) were given plants extracts at a dose of 5 and 10 mg/kg for each rat. Animals were then put into metabolic cages individually. Water and feed were available to the animals. Urine sample were collected and measured hourly for 4 hour. Urine density and urine pH were also measured every hourly. The content of urinary electrolytes that included sodium (Na^+), potassium (K^+) and chloride (Cl^-) was determined by using ion selective electrodes (ISE) automatic analyzer (Radiometer Copenhagen EML 100).

4.4 Serum analysis

After the diuretic experiments, rats were anesthetized using pentobarbitone (50mg/kg) and intra-cardiac blood samples (3 to 5 ml) were taken. Rats eventually sacrificed by cervical dislocation. Blood samples were centrifuged at 4000 rpm for 10 minutes. The serum was separated and frozen at -20^{o}C for analysis. Glucose, albumin, blood urea nitrogen (BUN) and creatinine level were analysed using an automatic analyzer (Hitachi Roche, 902 Automatic Analyzer).

4.5 Statistical Analysis

The results are presented as mean ± standard error of mean (S.E.M). All assessment data were analyzed using One-way analysis of variance (ANOVA) and result will be considered significant if ($p<0.05$). Duncan Test post hoc test was performed.

5. Results

5.1 Urine Volume

Table 1 exhibited the volume of urine of all treatment groups that collected every hour for 4 hours. *O. stamineus* extract with dose 5 and 10 mg/kg showed maximum output of urine during the last hour, 0.48 and 0.81 ml respectively. Positive control group, furosemide and hydrochlorothiazide provided the highest total volume. (Table 1). The results indicated that *O. stamineus* exhibited diuretic activities. *O. stamineus* extract at dose 10 mg/kg significantly increased total urine excreted for 4 hours with 16.60 ± 0.74 ml/kg. The diuretic action of *O. stamineus* was 15.51. However, for the positive control diuretic drugs furosemide and hydrochlorothiazide, the diuretic actions were 23.43 and 21.64 respectively (Table 1).

5.2 Urinary electrolytes

The amount of urinary sodium, potassium and chloride were measured every hour from the collected urine, as shown in Table 2. The urine content patterns of the three electrolytes of the negative control group were similar. The positive control groups; furosemide and hydrochlorothiazide, revealed that the amount of sodium and chloride were markedly increased every hour throughout the experiment. Interestingly, *O. stamineus* extract had reduced amount of the urine sodium compared

to the negative control group. *O. stamineus* extract however, showed markedly increased amount of the urine potassium every hour throughout the experiment compared to the negative control dose-dependently.

5.3 Urinary pH and Density

O. stamineus extract at both doses did not shows significant changes to the urine density or urine pH (Data not shown).

5.4 Blood serum analysis

The effect on glucose, albumin, BUN and creatinine concentration in rat's blood serum treated with aqueous extract of *O. stamineus* is summarized in Figure 1. The result showed that *O. stamineus* extract markedly increased the concentration of glucose, significantly compared to negative control. *O. stamineus* extract also markedly increase albumin, BUN and creatinine concentration, statistically significant relative to negative control.

6. Discussion

This study revealed that the water extracts from *O. stamineus* have a demonstrable diuretic activity. Furosemide caused the expected increase in the urine volume, renal excretion of Na^+ and K^+, whereas had relatively no effect on Cl^- loss. Furosemide acts by inhibiting electrolyte reabsorption in the thick, ascending limb of the loop of Henle (Johnson et al., 1999). On the other hand, hydrochlorothiazide enhanced urine output, Na^+ and Cl^- loss significantly. Hydrochlorothiazide inhibits sodium and chloride reabsorption in the distal tubules (Sripanidkulchai et al., 2001).

The diuretic action of *O. stamineus* extract was less potent than that of positive control; furosemide and hydrochlorothiazide.

Interestingly, our results revealed *O. stamineus* extract significantly increased excretion of potassium and chloride ions. *O. stamineus* extract did not give significant alteration of urinary levels of sodium. Mechanism of action of extracts is not similar to hydrochlorothiazide and furosemide. This diuretic action observed maybe depended on the stimulation of the urinary tract and which is linked to the activation of neurohumoral mechanism, mediators of stimuli acting on glomerules, tone acid on the pyelo-uretral peristaltis (Galati et al., 2002). The diuretic activity of *O. stamineus* may associate with the high salt or electrolyte content of its extract. These effects might be due to the influence that the electrolytes, present in considerable quantities on the plant, exert on renal epithelium (Galati et al., 2002). However, this activity appeared to not correlate well with the maximum urine volume and the amount of electrolytes excreted during the first hour of urine collection (Sripanidkulchai et al., 2001).

The plant extracts exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying anions (Pantoja et al., 1991; Bevevino et al., 1994). The other possibility for the observed diuretic properties could be due to direct action of potassium ion content of *O. stamineus* extracts caused by highest potassium ions contents (Ribeiro et al., 1988, Nilveses et al. 1989). Our results indicated that the existing diuretic activity of plant seemed to be mediated through a change in potassium transport. Plant extracts may be inhibiting potassium absorption or stimulating potassium secretion, or both, leading, in either case, to more potassium

retention in the lumen of the kidney tubules and osmotic water flow (Kreydiyyeh and Usta, 2002).

In this present study, the diuretic effect observed cannot exclude the possibility that changes in the diuresis may occur as a consequence of the presence of polar drug or active compounds, for examples flavonoid glycosides (Haloui *et al*., 2000), saponosids (Haloui *et al*., 2000) and ascorbic acid (Kuti, 1992; Haloui *et al.,* 2000). These natural compounds might be acting synergetically or individually promoting an initial vasodilatation (Stanic and Samarzˇija, 1993). It is also possible that plant extracts might manifest cumulative effect of several substances in the extract and/or due to secondary active metabolite (Tanira *et al*., 1988). Other monovalent and bivalent cations are present in this plant and might have a diuretic activity synergetically with K^+ (Galati *et al*., 2002).

Results from the presents study demonstrated various changes of concentration of the parameters in the blood serum analysis. There were significant changes occurred in the blood chemistry parameters, including glucose, creatinine, blood urea nitrogen (BUN), and albumin of treated animals group. Glucose level was slightly increased but not significantly for plants extracts. Even though, these results were statistically significant but these levels were still within normal range. In order to evaluate the biological significance, further studies are needed. As a conclusion, *O.stamineus* plant extracts exhibited diuretic properties which totally in agreement with the ethnomedical information that this plant is used for dysuria treatment. However, care must be taken when consume this herb as kidney function enzymes

were slightly elevated in the treated groups. This maybe more critical for patients with compromised kidney functions taking this herb.

Acknowledgments

This work was financially supported by the Ministry of Science, Technology and Innovations, Malaysia and Universiti Putra Malaysia.

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Figure 1: Mean concentration of glucose, albumin, Blood Urea Nitrogen (BUN) and creatinine of blood serum in rat groups treated with plants extract.

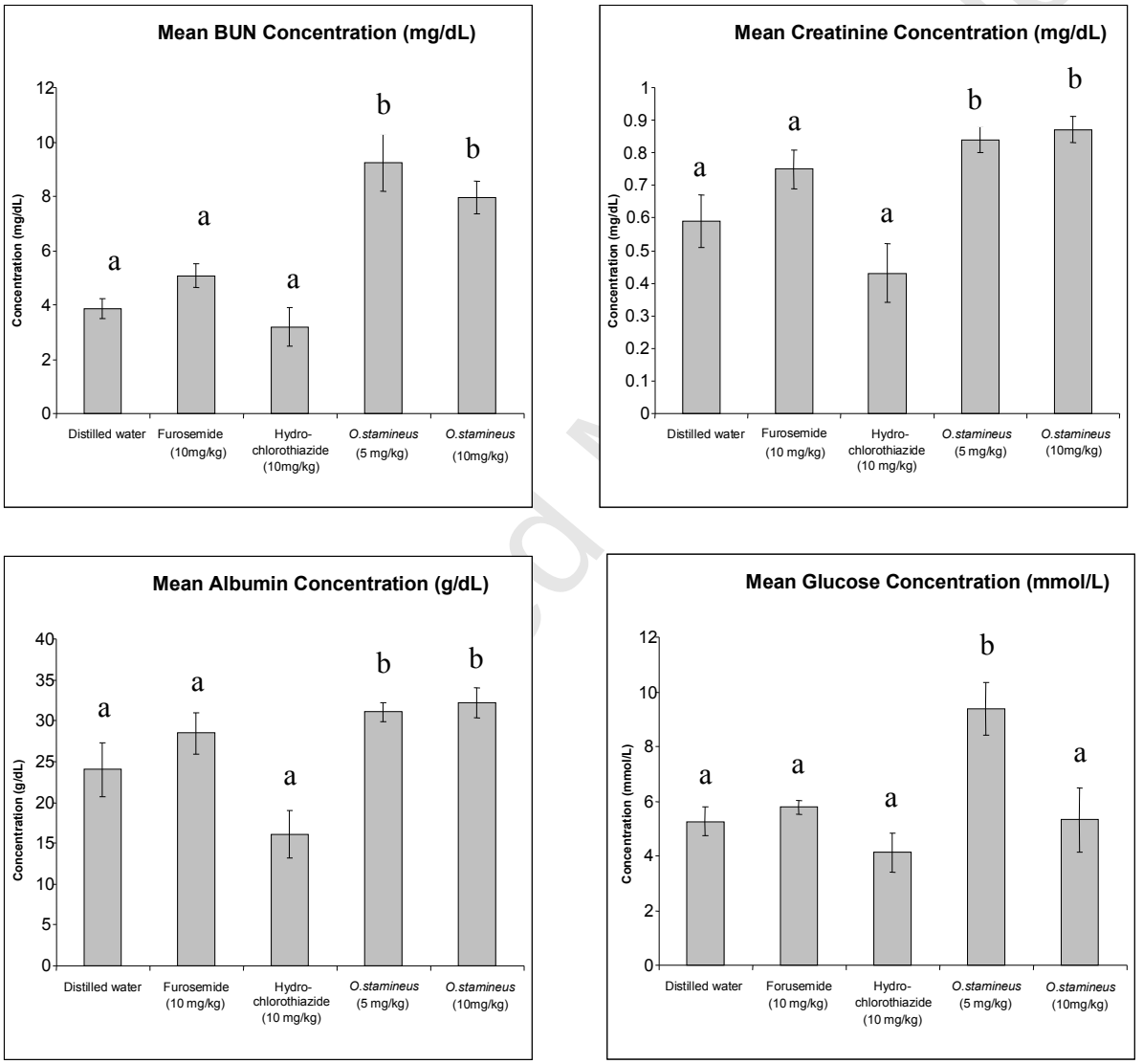

Table 1: Urine volume after the treatment with the plant extracts over a period of 4 hours

Treatment	Dose (mg/kg)	Urine Volume (ml)[1]					Total Urine vol (ml/kg BW in 4 Hr)	Diuretics action[3]
		Hr 1	Hr 2	Hr 3	Hr 4	Total		
Distilled water		0	0.05	0.12	0	0.17	1.07 ± 0.54[a]	1
Furosemide	10	0.75	1.07	0.76	0.48	3.06	25.07 ± 4.79[c]	23.43
Hydrochlorothiazide	10	0.10	1.10	0.66	0.68	2.55	23.16 ± 0.61[c]	21.64
O. stamineus	5	0	0.15	0.21	0.48	0.85	7.87 ± 0.43[a]	7.36
O. stamineus	10	0.16	0.33	0.57	0.81	1.87	16.60 ± 0.74[b]	15.51

[1] Each value represents the mean.
[2] Each value represents the mean ± S.E.M.
[3]Diuretics action = (urinary excretion of treated group (4 hr)) / (Urinary excretion of control group (4 h))
[a-c] Mean with different superscripts differ significantly at (P< 0.05)
BW=Bodyweight

Table 2: Total excretion over a period of 4 hour of urinary electrolytes

Treatment	Dose	Na^+ (mol)				K^+ (mol)				Cl^- (mol)			
	(mg/kg)	Hr1	Hr2	Hr3	Hr4	Hr1	Hr2	Hr3	Hr4	Hr1	Hr2	Hr3	Hr4
Distilled water		ND	17±0.1	20.5 ±1.22	ND	ND	67.3 ± 0.11	80.1±10.86	ND	ND	17.0 ± 0.32	31.0± 6.53	ND
Furosemide	10	139.67 ± 11.9	140 ± 3.21	117.33 ± 2.73	157.5 ± 8.57	142.3±21.47	77.3 ± 28.82	47.95±11.23	24.87± 3.74	188.0± 8.98	163.0 ± 33.4	133.5± 0.41	ND
Hydrochlorothiazide	10	159.0 ± 28.36	227.0±43.92	175.67±24.88	174.5±15.11	207.47±45.76	47.03 ± 10.23	38.8±4.16	85.6± 7.92	142.3±16.4	115.6± 11.3	110.0± 15.3	205.0± 22.86
O. stamineus	5	ND	35.0 ± 10.61	32.0 ± 19.03	11.0 ± 2.52	ND	385.1± 4.82	255.07±63.88	158.87± 26.98	ND	101.0 ± 23.6	68.67± 39.8	20.0± 5.13
O. stamineus	10	36.0 ± 0.15	18.5 ± 4.49	9.0 ± 1.63	5.33 ± 2.33	345.4± 9.67	593.2± 61.6	321.4±1.24	218.6± 96.91	63.0 ± 2.14	27.0 ± 7.35	31.5± 2.86	38.67± 9.94

Each value represents the mean ± S.E.M.
ND-Not determined (No urine)