Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 6 (5): 525-532, 2011
ISSN 1816-496X / DOI: 10.3923/jpt.2011.525.532
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Diuretic Activity of Leaves Extract of Hot Water Infusion of *Ruta graveolens* L. in Rats

[1]J.R.A.C. Jayakody, [1]W.D. Ratnasooriya, [1]W.A.N.A. Fernando and [2]K.R. Weerasekera
[1]Department of Zoology, University of Colombo, Colombo 03, Sri Lanka
[2]Department of Nidhana Chikitsa, Institute of Indigeneous Medicine, Rajagiriya, Sri Lanka

Corresponding Author: W.D. Ratnasooriya, Department of Zoology, University of Colombo, Colombo 03, Sri Lanka Tel: 0094 112503399 Fax: 0094 11 2503148

ABSTRACT

Ruta graveolens Linn (Family: Rutaceae) leaves are used in Sri Lankan traditional medicine as a diuretic but this effect is not scientifically validated. This study has evaluated the diuretic potential of *R. graveolens* leaves in rats using a Hot Water Infusion (HWI). Different concentrations of HWI (4.5, 6.75, 9.0 mg mL^{-1}) or vehicle or furosemide (13 mg kg^{-1}) were orally administrated (n = 6 per group) to hydrated rats and their urine output was monitored hourly for 6 h. Urinary pH, specific conductivity, specific gravity, Na^+, K^+, Cl^- levels and creatinine clearance were determined (with the highest dose and control). Using these data standard urinary indices were calculated. Further, subchronic toxicity was examined in terms of serum Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT), urea and creatinine levels and overt signs. HWI increased the urine output markedly in a dose-dependent manner. The high dose of HWI was almost equipotent to furosemide (in terms of diuretic activity). The onset of diuresis was very rapid (within 1 h) and lasted throughout the studied period. HWI also caused significant increase in specific gravity, specific conductivity, creatinine clearance, Na^+ and K^+ levels, thiazide secretion index, urine alkaline index, diuretic action, Na^+ and K^+ saliuretic indices and significant decrease in carbonic anhydrase index. Further, no evidence of subchronic toxicity was seen. *R.graveleons* leaves exhibits safe and strong oral diuretic activity as claimed in Sri Lanka traditional medicine. This action is mediated via multiple mechanisms: thiazide like activity, inhibition of carbonic anhydrase activity and increase in glomerular filtration rate.

Key words: *Ruta graveleons,* traditional medicine, diuretic, toxicity, hot water infusion

INTRODUCTION

Ruta graveolens Linn (Family: Rutaceae), Aruda in Sinhala, Aruvadam in Tamil and Garden Rue in English is a perennial semi-shrubby plant, 65-75 cm tall, with a sharp unpleasant odour. The stem is very ramified. Its leaves are green to strongly alaucos blue-green in colour, alternate, bipinnate or tripinnate with a feathery appearance. The flowers are yellow, regular, bisexual, long stalked with 4-5 petals and born in cymes. The fruit is a 4-5 lobed capsule containing numerous seeds. The plant is native to Europe and is cultivated in India and in the up-country in Sri Lanka (Jayaweera, 2006). Phytochemically, the leaves of *R. graveolens* reported to contain appreciable amounts of glycosides (such as rutine) variety of flavonoids, alkaloids (such as coquisagenine, skimmianine, graveoline), furocoumarins (such as bergaptene, xantotoxine), a variety of essential oils (Lemonin) and alcohols (such as methyl-ethyl-carbinol) (De Feo *et al*., 2002).

In traditional and folk medicine of several countries it is claimed to be prescribed alone or in combination as a promising treatment for several diseases and disorders. These includes insomnia, headaches, nervousness, epilepsy, hysteria, abdominal cramps, certain eczemas and psoriasis (EI-Agraa *et al*., 2002; Bohidar *et al*., 2008). The infusions are also used as an emmenagogue, to induce abortions (Ciganda and Laborde, 2003) and to treat certain diseases of womb (EI-Sanusi and EI-Adam, 2007) and in Taiwan, also in the treatment of palpitation and heart protection (Seak and Lin, 2007).

Experimentally, petroleum ether, chloroform (El-Sayed *et al*., 2000) and methanol (Ratheesh and Helen, 2007) extracts of arial parts of this plant have been shown to possess marked anti-inflammatory action when tested against rat carregeenan-induced paw edema model. On the other hand, hot water extract of the whole plant has been shown to suppresses male sexual competence and fertility in rats (Khouri and El-Akawi, 2005) and methanolic extract to impair fertilizability of rat sperm *in-vitro* (Rahim *et al*., 2010).

In Sri Lankan traditional medicine, in addition to some of these reported uses it is also claimed that the sharp smell of the leaves of *R. graveolens* promotes sexual desire in women and leaf infusion possesses potent diuretic activity (Weragoda, 1994). Interestingly, diuretic activity of this plant is also claimed in traditional medicine in Jordan (Khouri and El-Akawi, 2005). However, these two claimed activities are not tested and validated by scientifically controlled experiments. This study was therefore undertaken to investigate the diuretic potential of hot water infusion (HWI) of *R.graveolens* leaves using conscious hydrated rats.

MATERIAL AND METHODS

Experimental animals: Healthy, adult crossbred male albino rats weighing 200-225 g from our own colony were used (n = 42). They were housed in standard environmental conditions (temperature: 28-31°C, photoperiod: approximately 12 h natural light per day, relative humidity: 50-55%). The animals were fed with pelleted food (Ceylon Grain Elevators, Colombo, Sri Lanka) and clear drinking water *ad libitum*. Except at the time of experimental procedure the animals were handle only during cage cleaning. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka, for animal experimentations.

Collection of leaves: Fresh mature leaves were plucked from a *R. graveolens* tree from a home garden at Bandarawela (Uva, province 1230 m from sea level), Sri Lanka, in March 2009. These were identified and authenticated by Prof. (Mrs.) A. N. Seneviratne, Department of Plant Science, University of Colombo, Sri Lanka. A voucher specimen (WDR 01/2010) is deposited at the museum of the Department of Zoology, University of Colombo, Sri Lanka.

Preparation of the Hot Water Infusion (HWI) of *R. graveolens*: The leaves were shade dried (28-30°C) for 7 consecutive days. 1.8 g of the dried leaves were socked in 20 mL of boiling water for 30 min. The resulting straw colored infusion was filtered through a muslin cloth (yield 42.2% w/w). Three doses of HWI (9.00, 6.75 and 4.5 mg mL^{-1} HWI in 1 mL) was made by appropriate dilution and used within 30 min. The highest dose selected is ten times higher than the normally recommended by the traditional practitioners which is within the accepted range for the rat model (Dhawan and Sirimal, 2000).

Evaluation of diuretic activity: Thirty rats were deprived of water but not food for 18 h. Their urinary bladders were emptied by gentle compression of the pelvic area and by pull of their tails. Each of these rats was then orally administered with 15 mL of isotonic saline (NaCl, 0.9 % w/v) to impose a uniform water load. Forty-five minutes later, these rats were randomly assigned in to five groups (n = 6 per group) and treated orally in the following manner (Group 1) 1 mL of water, (Group 2) 4.5 mg mL^{-1} of HWI, (Group 3) 6.75 mg mL^{-1} of HWI, (Group 4) 9.00 mg mL^{-1} of HWI and (Group 5) 13 mg kg^{-1} of furosemide (State pharmaceutical Corporation, Colombo, Sri Lanka) the reference drug (Rang *et al*., 2003; Dharmasiri *et al*., 2003). Each of these rats was individually placed in metabolic cages and cumulative urine output was determined at hourly intervals for 6 h. The color of urine was also noted.

In an attempt to ascertain the broad mechanisms of diuretic action, the urine collected from group 1 (control) and group 4 (9.00 mg mL^{-1} of HWI) were subjected to the following investigations: pH (by pH meter, Toa electronics Ltd., Tokyo, Japan), Na^+, K^+ and Cl^- levels by flame photometry (compact atomic absorption spectrophotometer, GFS Scientific Equipment Pvt. Ltd., Sydney, Australia), specific gravity, specific conductivity, glucose and proteins (using Combistrix®, reagent strips, Bayer Diagnostics Manufacturing Ltd., Bridgent, UK). Na^+/K^+ (aldosterone secretion index), Na^+/Cl^- (thiozide secretion index), Na^+/H^+ (urine alkaline index), $Cl^-/Na^+ + K^+$ (carbonic anhydrase index), diuretic action (urinary output of treated group/urinary output of control group) and diuretic activity or potency (urinary output of treated group/urinary output of furosemide treated group) ratios were computed. Saliuretic index for Na^+ (Na^+ in treated group/Na^+ in control group), K^+ (K^+ in treated group/K^+ in control group) and Cl^- (Cl^- in treated group/Cl^- in control group) were also calculated (Durairaj *et al*., 2007; Somova *et al*., 2003; Junior *et al*., 2009; Lahlou *et al*., 2007; Wright *et al*., 2007).

Evaluation of Na^+ and K^+ in HWI: Na^+ and K^+ levels in the highest dose of HWI were measured by flame photometry as described previously.

Estimation of creatinine clearance: Twelve rats were randomly divided into two equal groups (n = 6/group), fasted and hydrated as described previously. One group was orally administrated with 1 mL of water and the other with 9.0 mg mL^{-1} of HWI. These rats were individually placed in metabolic cages and their cumulative urine output was measured after 2 and 24 h. Blood was also collected from tails using aseptic precautions and serum was separated. Creatinine levels in the urine and serum were determined using Randox kits (Randox Laboratories Ltd., Antrim, UK). Creatinine clearance was computed as per instructions given by the manufacturer using the data obtained. Creatinine clearance was taken as an estimation of the glomerular filtration rate (Ratnasooriya *et al*., 2009).

Evaluation of acute and subchronic toxicity: Twelve male rats were randomly assigned into two equal groups (n = 6/group). The first group was orally treated for 30 consecutive days (at 9.00 h) with the 9.00 mg mL^{-1} of HWI and the other with 1 mL of water. During this period, each rat was observed for the following. Overt signs of toxicity (salivation, lachrymation, breathing distress, ptosis, stupor, squint, teeth exposure, writhing, convulsion, tremors, yellowing of fur and loss of fur), Stress (erection of fur and exophthalmia), Behavioural abnormalities (such as impairment of spontaneous movements, climbing, cleaning of face, ataxia, rolling and other postural changes) and aversive behaviours (biting and scratching, licking of tail, paw and penis,

intense grooming or vocalization) and diarrhoea. On day 1 post treatment (31st day), these rats were anaesthetized with ether and blood was collected from tails using aseptic precautions. Estimation of serum urea and creatinine (to examine renal toxicity), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) (to examine liver toxicity) were made using respective kits (Randox Laboratories Ltd., Antrim, UK).

Statistical analysis: Data are represented as Mean±SEM Statistical comparisons were made by one way ANOVA followed Turkey's post hoc test and Ma.nn-Whitney U-test (Bluman, 1998) as appropriate using minitab 13.0 version statistical package. Significance was set at $p<0.05$.

RESULTS

Evaluation of diuretic activity: As shown in Table 1, mid dose (6.75 mg mL^{-1}), (high dose 9.00 mg mL^{-1}) of HWI and reference drug furosemide (13 mg kg^{-1}) significantly ($p<0.05$) and markedly increased the cumulative urine output, at 6 h. On the other hand, low dose 4.5 mg mL^{-1} of HWI although increased the urinary output by slightly the effect was not significant ($p>0.05$). In addition, linear regression analysis revealed that the diuretic effect was dose-dependent ($r = 1.0$, $p<0.05$). The onset of diuresis by HWI was evident at 1 h and lasted up to 6 h. with mid (6.75 mg mL^{-1}) and high (9.00 mg mL^{-1}) doses (Fig. 1). On the other hand, with furosemide both the onset and peak diuresis occurred at 1 h and there was no significant difference ($p>0.05$) in the cumulative urine out put between the high dose (9.00 mg mL^{-1}) of HWI. Further, with furosemide, on hourly basis, significant ($p<0.05$) diuresis was seen only at 1st h.

As shown in Table 2, the highest dose (9.00 mg mL^{-1}) of HWI significantly ($p<0.05$) increased the specific gravity, specific conductivity Na^+ level, K^+ level, Na^+/H^+ ratio and Na^+/Cl^- ratio of urine. It also slightly but significantly ($p<0.05$) suppressed the urinary H^+ level. In contrast, the highest dose of HWI significantly ($p<0.05$) decreased the urinary $Cl^-/Na^+ + K^+$ ratio. Further, the highest dose (9.00 mg mL^{-1}) of HWI markedly increased the sodium and potassium saliuretic indices and diuretic action. On the other hand, the other urinary parameters determined (pH, H^+ level, Cl^- level, Na^+/K^+ ratio) and computed with the highest dose (9.00 mg mL^{-1}) of HWI were not significantly ($p>0.05$) altered.

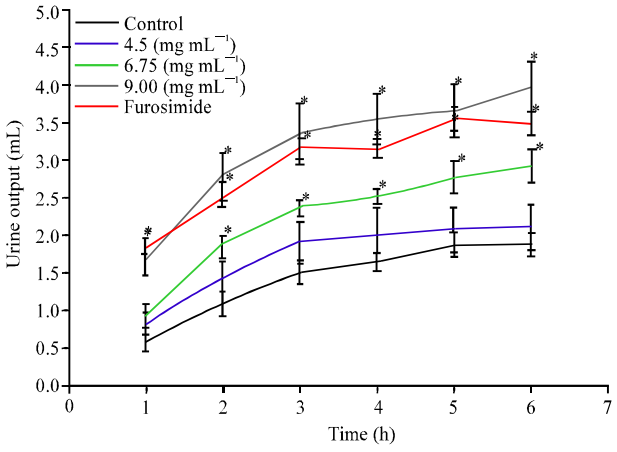

Fig. 1: Time course of diuresis in rats treated with (4.50, 6.75 or 9.00 mg mL^{-1}) different doses of Hot Water Infusion (HWI) of *Ruta graveolens*, vehicle and reference drug, Furosimide. Each point represent the mean of 6 rats and vertical bars indicate (Mean±SEM) *$p<0.05$

Table 1: Cumulative urine output in rats over a 6 h period following oral administration of Hot Water Infusion (HWI) of *Ruta graveolens* (Mean±SEM) n = 6

Treatment	Total Urine output (mL/100 g /b.wt./h
Control	1.88
4.50 mg mL^{-1} of HWI	2.11
6.75 mg mL^{-1} of HWI	2.93*
9.00 mg mL^{-1} of HWI	3.90*
13 mg kg^{-1} of Furosemide	3.64*

*p<0.05 as compared with control ANOVA- followed by Turkey's post hoc test

Table 2: Effect of orally administrated hot water infusion HWI of *Ruta graveolens* (9.00 mg mL^{-1}) on some urine parameters (up to 6 h) of rats (Mean±SEM) n = 6

Parameter	Control group	Treated group (9.0 mg mL^{-1})
pH	7.08±0.30	6.66±0.16
H^+ (ppm)	0.8504±0.017	0.8113±0.005*
Specific gravity	1.010±0.00	1.024±0.00*
Conductivity (ms)	14.99±0.37	29.76±1.06*
Na^+ (ppm)	4625±259	8644±193*
K^+ (ppm)	2320±148	5072±171*
Cl^- (ppm)	7384±213	7898±731
Na^+/K^+	1.081±0.055	0.5764±0.050
Na^+/Cl^-	0.631±0.045	1.128±0.091*
Na^+/H^+	5643.25±368.45	10654.07±235.49*
Sodium saliuretic index	1.0	1.86
Potassium saliuretic index	1.0	2.19
Chloride saliuretic index	1.0	1.07
Diuretic index	1.0	1.98
Diuretic activity (potency)	-	1.186

*p<0.05 as compared with control (Mann-Whitney, U-test)

Table 3: Effect of orally administered hot water infusion HWI of *Ruta graveolens* on some serum bio chemical parameters of rats (Mean±SEM) n = 6

Parameter	Control	Treatment
SGOT (UL^{-1})	27.11±2.25	28.00±4.60
SGPT (UL^{-1})	16.00±0.45	12.00±1.30
Urea (mg dL^{-1})	24.51±2.41	22.66±3.70
Creatinine (mg dL^{-1})	2.15±0.32	2.52±0.36

Evaluation of creatinine clearance: The high dose of HWI induced a pronounced and significant (p<0.05) increase (by 61%) in glomerular filtration rate at 1h as examined by creatinine clearance.

Evaluation of acute and chronic toxicity: No deaths were encountered with subchronic treatment of the high dose of HWI. The high dose (9.00 mg mL^{-1}) of HWI did not induce any overt signs of toxicity, stress, behavioural abnormalities or aversive behaviours. Further, none of the serum parameters (SGOT, SGPT, Urea and Creatinine levels) investigated was significantly (p>0.05) altered (Table 3).

Evaluation of Na^+ and K^+ in HWI: Na^+ and K^+ level in HWI was respectively 23.8±0.19 (ppm) and 681.84±4.98 (ppm).

DISCUSSION

This study examined the oral diuretic potential of *R. graveolens* leaves in conscious rats using HWI. The results showed, for the first time, that *R. graveolens* leaves possess strong diuretic (in terms of cumulative urine output and diuretic action), hypernatiremic (in terms of urinary Na^+ level and sodium saliuretic index) and hyperkalemic (in terms of urinary K^+ level and potassium saliuretic index) activities. The diuretic efficacy of HWI was almost comparable (diuretic activity 0.86) to furosemide, widely used synthetic loop diuretic in clinical practice (Anonymous, 2001). The onset of diuretic activity of HWI was extremely rapid (1-2 h), almost similar to furosemide, indicating quick absorption from the gastrointestinal tract. Further, the rapid onset of diuresis by HWI indicates that its diuretic action is unlikely to be mediated via a secondary organic metabolite. The diuretic activity of HWI lasted throughout the study period (up to 6 h) suggesting a slow clearance. Such an action profile is therapeutically desirable. Interestingly, the diuretic action of HWI was dose-dependent indicating that this effect is intrinsic, genuine and causal and possibly receptor mediated. However, even the receptors for clinically most important diuretics are yet unknown (Odlind, 1984).

Some herbal diuretics induce diuresis by stimulating the thirst centre in the hypothalamus and thereby enhancing the fluid intake (Odlind, 1984; Neuman, 2002). Such a mode of action is unlikely in this study as the rats had no access to water during the study period. The onset of diuretic action of HWI was quick, the effect was strong and the urine was markedly hyperkalemic (in terms of urinary K^+ content and potassium saliuretic index). Further, the HWI neither increased the Na^+/K^+ ratio (aldesterone secretory index) (Durairaj *et al.*, 2007) nor caused alkalization of urine. These observation collectively suggest that HWI is not acting as a potassium-sparing diuretic: potassium-sparing diuretics are usually very weak has a slow onset of action, causes urine alkalization and increase the urinary Na^+/K^+ ratio (Dharmasiri *et al.*, 2003; Anonymous, 2001; Jayakody and Ratnasooriya, 2005). Some botanicals are claimed to mediate diuresis, at least partly, by vasodilation (Martin-Herrera *et al.*, 2008) increasing renal blood flow (Martin-Herrera *et al.*, 2008), uterine peristalsis (Wright *et al.*, 2007) or number of functional glomeruli in the kidneys (Wright *et al.*, 2007). However, at present, we do not have evidence in favour or against the operation of such potential mechanisms with HWI.

HWI provoked an increase in urinary specific gravity and urinary specific conductivity which is an indirect measure of urinary ionic content (Abdala *et al.*, 2008). This suggests that osmotic type of mechanism may play a role, at least partly, in inducing diuresis. However, it is not due to high levels of Na^+ and K^+ ions in the HWI; since the levels of these two ions in HWI is far below that is required to induce salt diuresis (Sripanidkulchai *et al.*, 2001). The urine of HWI treated rats was both markedly hypernatremic (in terms of urinary Na^+ level and sodium saliuretic index) and hyperkalemic (in terms of urinary K^+ level and potassium saliuretic index) but not hyperchloremic (in terms of urinary Cl^- level and chloride saliuretic index). Further, HWI increased the urinary Na^+/Cl^- ratio [thiazide secretory index (Durairaj *et al.*, 2007)] significantly without causing alkalization (in terms of urinary pH) of urine. Collectively, these observations suggest a thiazide like mode of diuretic action of HWI. Thiazide type of diuretics elevate thiazide secreatory index, simultaneously increase urinary Na^+ and K^+ levels at least by 50-60% by inhibiting the Na^+/Cl^- co-transpoter in the distal convoluted tubule of the nephron (Lahlou *et al.*, 2007).

On the otherhand, in addition to above features, the diuretic action of HWI was extremely rapid (within 1-2 h), strong (in terms of cumulative urine output and diuretic index), potent (in term of diuretic action) and exhibited a diuretic profile (Fig. 1) almost identical to furosemide, a high celing diuretic which acts by inhibiting the $Na^+/K^+/2Cl^-$ co-transporter in the thick region of the ascending limb of loop of Henle (Rang *et al*., 2003; Lahlou *et al*., 2007). This suggests that HWI may also have a loop diuretic type of mode of action. However, loop diuretics usually increase urinary Cl^- level (Lahlou *et al*., 2007; Anonymous, 2001) but such a feature was not evident in this study. Nevertheless, it is of interest to note that in rats, even furosemide, a high celing loop diuretics does not always increase urinary Na^+ content (Lahlou *et al*., 2007).

The HWI of *R. graveolens* leaves provoked a huge and a significant impairment in urinary $Cl^-/Na^+ + K^+$ ratio [(carbonic anhydrase index) (Durairaj *et al*., 2007)]. This is suggestive of inhibition of carbonic anhydrase enzyme in inducing diuresis. HWI also profoundly increased the glomerular filtration rate (in terms of creatinine clearance). This is yet another mechanism of promoting diuresis by HWI. Thus, HWI appears to have a multiple mode of diuretic action. This is possible since HWI contains a variety of phytoconstituents such as alkoloids, phenolic compounds, flavonoids, saponins and volatiles (De Feo *et al*., 2002) which are endowed with diuretic properties (Chandra *et al*., 2008). Multiple mode of diuretic action is reported with some herbal medications (Wright *et al*., 2007; Chandra *et al*., 2008).

The HWI was well tolerated with an encouraging safety profile (as judged by absence of mortality, morbidity, overt signs of toxicity, stress, behavioural abnormalities and increased levels of serum GOT, GPT, creatinine and urea) In contrast, several undesirable side effects are reported with many of the currently used diuretics (Anonymous, 2001; Koti and Purnima, 2008).

In conclusion, this study provides first scientific evidence in favour of claimed diuretic potential of *R. graveolens* leaves in Sri Lankan traditional ethnomedicine. It further shows that HWI of *R. graveolens* leaves has a multiple mode of diuretic action.

REFERENCES

Abdala, S., D. Martin-Herrera, D. Benjumea and P. Perez-Paz, 2008. Diuretic activity of *Smilax canariensis*, an endemic Canary island species. J. Ethnopharmacol., 119: 12-16.

Anonymous, 2001. British National Formulary. British Medical Association and the Royal Pharmacological Society of Grate Britain, London, pp: 63-69.

Bluman, A.G., 1998. Elementary Statistics. 3rd Edn., Addison Wesley Longman, Califonia, USA., pp: 599-623.

Bohidar, S., M. Thirunavoukkarasu and T.V. Rao, 2008. Effect of plant growth regulators on in vitro micropropagation og garden rue' (*Ruta graveolens* L.). Int. J. Int. Biol., 3: 36-43.

Chandra, P.K., I.J. Kuppast, C. Manjunath, N. Jawahar, S. Jubieand and B. wapna, 2008. Diuretic activity of whole plant extracts of *Cardiospermum halicacabum* (Linn). Phcog. Mag., 4: 80-82.

Ciganda, C. and A. Laborde, 2003. Herbal infusions used for induced abortion. Clin. Toxical., 41: 235-239.

De Feo, V., F. De Simone and F. Senatore, 2002. Potential allelochemicals from the essential oil of *Ruta graveolens*. Phytochemistry, 61: 573-578.

Dharmasiri, M.G., W.D. Ratnasooriya and M.I. Thabrew, 2003. Diuretic activity of leaf and stem decoction of *Anisomeles indica*. J. Trop. Med. Plants, 4: 43-45.

Dhawan, B.N. and R.C. Sirimal, 2000. Acute Toxicity and Gross Effects. Laboratory Manual for Pharmacological Evaluation of Natural Product ICS Ave Science Park, Italy, pp: 17-18.

Durairaj, A.K., U.K. Mazumder, M. Gupta and S.K. Ray, 2007. Effects of methonolic extract of *Oxystelma esculentum* on diuresis and urinary electrolytes excretion in rats. Iranian J. Pharmacol. Therp., 6: 207-211.

El-Agraa, S.E., S.M. El-Badwi and S.E. Adam, 2002. Preliminary observation on experimental *Ruta graveolens* toxicosis in Nubian goats. Trop. Anim. Health Prod., 34: 271-281.

El-Sanusi, N.I. and S. EI-Adam, 2007. The effect of low levels of dietary *Ruta graveolens* and Solenostemma argel or their mixture on bovans chicks. Asian J. Anim. Vet. Adv., 2: 27-31.

El-Sayed, N.H., N.M. Ammar, S.Y. Alokbi, L.T.A. El-Kassemb and T.J. Mabry, 2000. Bioactive chemical constituents from *Ruta graveolens*. Latinoamericana de Quimica, 28: 61-64.

Jayakody, J.R.A.C. and W.D. Ratnasooriya, 2004. Diuretic activity of hot flower infusion of *Nyctanthes arbo-tristis* in rats. BLACPMA., 3: 84-87.

Jayaweera, D.M.A., 2006. Medicinal Plants (Indigenous and Exotic) Used in Ceylon. 5th Edn., National Science Foundation, Sri Lanka, pp: 35-37.

Junior, A.G., M.A. Boffo, E.L.B. Lourenco, M.E.A. Stefanello, C.A.L. Kassuya and M.C.A. Marques, 2009. Natriuretic and diuretic effects of *Tropaeolum* (Tropaeolaceae) in rats. J. Ethnopharmacol., 122: 517-522.

Khouri, N.A. and Z. El-Akawi, 2005. Antiandrogenic activity of *Ruta graveolens* L. in male Albino rats with emphasis on sexual and aggressive behavior. Neuro Endocrinol. Lett., 26: 823-829.

Koti, B.C. and A. Purnima, 2008. Diuretic activity of extracts of *Centratherum anthelminticum*. Int. J. Green Pharmacy, 2: 228-231.

Lahlou, S., A. Tahraoui, Z. Israili and B. Lyoussi, 2007. Diuretic activity of the aqueous, extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. J. Ethnopharmacol., 110: 458-463.

Martin-Herrera, D., S. Abdala, D. Benjumea and J. Gutierrez-Luis, 2008. Diuretic activity of some *Withania aristata* Ait. fractions. J. Ethnopharmacol., 117: 496-499.

Neuman, M., 2002. Metabolic effects and drug interactions provoked by certain vegetables: Grapefruit, St. John's wort and garlic. Presse Med., 31: 1416-1422.

Odlind, B., 1984. Site and mechanism of the action of diuretics. Acta Pharmacol. Toxicol., 54: 5-15.

Rahim, F., G. Saki and M. Bazrafkan, 2010. Effect of alcohol extracts of the *Ruta graveolens* L. on the count, motility and *in vitro* fertilization capacity of rat's sperm. Asian J. Plant Sci., 9: 63-66.

Rang, H.P., M.M. Dale, J.M., Ritter and P.K. Moore, 2003. Pharmacology. 5th Edn., Churchill Livingstone, Edinburgh, pp: 350-366.

Ratheesh, M. and A. Helen, 2007. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. Afr. J. Biotechnol., 6: 1209-1211.

Ratnasooriya, W.D., T.S.P. Fernando and R.A.A.R. Ranatunga, 2009. Diuretic activity of Sri Lankan black tea (*Camellia sinensis* L.) in rats. Phcog. Mag., 1: 4-10.

Seak, C.J. and C.C. Lin, 2007. Ruta Graveolens intoxication. Clin. Toxicol., 45: 173-175.

Somova, L.I., F.O. Shode, P. Ramanan and A. Nadar, 2003. Antihypertensive, antiatherosclerotic and antioxident activity of triterpenoids isolated from *Olea europaea*, subspecies Africana leaves. J. Ethnopharmacol., 84: 299-305.

Sripanidkulchai, B., V. Wonqpanich, P. Laupattarakasem, J. Suwansakri and D. Jirakulsomchok, 2001. Diuretic effects of selected Thai indigenous medicinal plants in rats. J. Ethnopharmacol., 75: 185-190.

Weragoda, P.B., 1994. Osuturu Visituru. 1st Edn., Department of Ayurveda, Colombo, Sri Lanka, pp: 79-80.

Wright, C.I., L. van Buren, C.I. Kroner and M.M. Koning, 2007. Herbal medicines as diuretics: A review of the scientific evidence. J. Ethnopharmacol., 114: 1-31.