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Diuretic activity of Sri Lankan black tea (*Camellia sinensis* L.) in rats

W.D. Ratnasooriya*, T.S.P. Fernando and R.A.A.R Ranatunga

Department of Zoology, University of Colombo, Colombo 03, Sri Lanka.

** Author of correspondence: Professor W.D. Ratnasooriya : E-mail: wdr@zoology.cmb.ac.lk*

ABSTRACT

The aim of this study was to evaluate the diuretic potential of Sri Lankan black tea (*Camellia sinensis* L.). This was assessed in rats using high grown Dust grade No: 1 tea, which is consumed widely by the tea drinkers worldwide. Different doses of hot black tea brew (BTB) (84, 167, 501 or 1336 mg/ml respectively equivalent to 1.5, 3, 9 and 24 cups) were made and orally administered to previously starved (24 h) but subsequently hydrated (with 15 ml of isotonic saline) rats and their urinary output was monitored cumulatively at hourly intervals for 6h. The reference drug used was frusemide (13 mg/kg). The results showed that BTB induced significant ($P < 0.05$), mild to moderate and dose- dependent diuresis (starting from 167 mg/ml). This diuretic activity had a fairly rapid onset (within 2 h) and relatively short duration of action (3 h). BTB also significantly ($P < 0.05$) increased the overall urinary frequency. Further, the diuretic activity of BTB was less potent to frusemide (by 45%). Decaffeination of black tea almost completely abolished the diuresis. The diuresis of the BTB was solely due to increased (by 55 %) urinary Na^+ excretion (with no urinary K^+ loss). Further, the chronic daily administration of the BTB did not develop tolerance or induce toxicity (general, renal and hepatic). It is concluded that BTB made from Sri Lankan high grown Dust grade No :1 tea has safe, mild to moderate diuretic activity with rapid onset and relatively short duration of action. Further, this study supports the claim made by Sri Lankan indigenous physicians that it is a diuretic.

KEYWORDS: *Camellia sinensis*, black tea, diuresis, Sri Lankan tea, dust grade tea

INTRODUCTION

Regular consumption of black tea brew (BTB) of *Camellia sinensis* (L.) O.Kuntz (family: Theaceae) on a daily basis (1.5 to 3 cups) is recommended by traditional physicians practicing in Polonnaruwa district (in the dry zone), North Central province of Sri Lanka to promote urinary flushing (1) possibly by acting as a diuretic. Indeed, some studies have demonstrated diuretic potential of BTB of *C. sinensis* (2,3). In these studies, the origin of the black tea is however not specified. In contrast, in a preliminary study, conducted in Sri Lanka by Goonaratna et al. (4), using a single dose of BTB (possibly made from black tea of Sri Lanka), have failed to demonstrate any diuretic activity (in terms of urinary volume, osmolality, sodium and potassium levels). This result cannot be simply overlooked and needs validation. This reportedly insignificant diuretic activity of BTB of Sri Lankan *C. sinensis* is possible, since it is known that several factors such as the country of origin, the geological background of the soil, the elevation of the tea plantation, the collecting season, technological processes during tea production and brewing conditions affects the final composition of BTB (5, 6, 7) and hence its pharmacological effects. BTB is claimed to possess antimalarial activity in traditional medicine (8) but a recent experimental study conducted using murine models has failed to show antimalarial potential, of BTB made from Sri Lankan tea, atleast against, *Plasmodium falciparum* (9). Therefore, reinvestigation of diuretic potential of Sri Lankan black tea was taken up.

The main aim of this study was to assess the diuretic potential of Sri Lankan black tea using several doses and to elucidate its broad mechanisms of action in the rat model using high grown Dust grade No: 1 tea (most widely used BTB in Sri Lanka). The other aim was to investigate the toxic effects of BTB following its chronic oral administration.

METHODS AND MATERIALS

Experimental Animals

Healthy adult Wistar rats (males 170-200 g and females 165-210 g) purchased from Medical Research Institute, Colombo, Sri Lanka were used. They were kept under standardized animal house conditions (temperature: 28-31 ^{o}C, photoperiod: approximately 12 hours natural light per day, relative humidity: 50-55%) at the animal house of the Department of Zoology University of Colombo. All rats had free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and domestic tap water. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka, for animal experimentations.

Source of tea

Topmost immature leaves and buds of *C. sinensis* plucked from the plantation of St. Coombs tea estate of the Tea Research Institute, Talawakelle, Sri Lanka (1382 m above sea level: high grown) in August 2005 were used to process Dust grade No: 1 black tea by orthodox-rotorvane technique at the estate factory. The tea sample was pure, unblend and typical to the grade as confirmed by sieve analysis, organoleptic profile, and physical and chemical analysis. Tea samples were packed in triple laminated aluminium foil bags (1 kg each) and stored at - 20^{0}C until use. The *C. sinensis* leaves used to manufacture the black tea samples was identified and authenticated by Professor (Mrs.) A.S. Senaviratna, Department of Plant Science, University of Colombo. A voucher specimen (wdr/tspf 200) was deposited at the museum of the Department of Zoology University of Colombo. Decaffeinated tea was made as described by Pavia et al (10).

Preparation of tea brew(BTB)

BTB was made according to the ISO standards (11) adding 2g of black tea to 100 ml of boiling water and brewing for 5 min. This contains 43.7% (w/w) tea solids in water as reported previously (12). Based on this data, 1336 mg/ml (equivalent to 24 cups, 1 cup = 170 ml) of BTB in 2 ml was made by adding 8g black tea to 15 ml boiling water and brewing for 5 min.501 mg/ml (equivalent to 9 cups), 167 mg/ml (equivalent to 3 cups) and 84 mg/ml (equivalent to 1.5 cups) concentrations of BTB were then made by diluting appropriately with boiling water.

Evaluation of the diuretic activity

126 (males 64 and female 62) rats were deprived of water but not food for 24 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 gavaged with 15 ml of isotonic saline (0.9% NaCl) to impose a uniform hydration status. One hour later, these rats were assigned into 7 groups and treated orally in the following manner: Group 1 (n = 27) 2 ml of water; Group 2 (n = 19) 84 mg/ml (low dose, equivalent to 1.5 cups) of BTB; Group 3 (n = 18) 167 mg/ml (mid dose, equivalent to 3 cups) of BTB; Group 4 (n = 25) 501 mg/ml (high dose, equivalent to 9 cups) of BTB; Group 5 (n = 21) 1336 mg/ml (supraphysiological dose, equivalent to 24 cups) of BTB; Group 6 (n = 8) 167 mg/ml (mid does, equivalent to 3 cups) of decaffeinated BTB and Group 7 (n = 8) 13 mg/kg of frusemide(13), a potent diuretic drug (State Pharmaceutical Corporation, Colombo, Sri Lanka). Each rat was then individually placed in a specially made metabolic cage (Jayawardena Fabricators Colombo, Sri Lanka) and the cumulative urine output was determined at hourly intervals for 6 h (13). During this period these rats had no access to water and food. The colour of urine was also noted.

To investigate whether tolerance developed to BTB induced diuresis with subchronic administration, 12 rats were randomly divided into two equal groups (n = 6/group). One group was orally treated with 2 ml water daily (at 9.00 h) for 28 consecutive days and the other with 167 mg/ml of BTB. After 14 and 28 days of treatment, these rats were fasted and hydrated as described previously and rats were individually placed in metabolic cages and their urine output measured cumulatively up to 6 h.

Evaluation of urinary frequency

This was done in two groups of rats made hydrated as in the previous investigation. One group (n = 9) was orally administered with 2 ml of water and the other group (n = 9) with the 167 mg/ml of BTB. These rats were individually placed in metabolic cages and the number of times each rat urinated per hour was observed for 6 hs.

Urine analysis

In an attempt to ascertain the broad mechanism of action of BTB induced diuresis, 12 rats were randomly

divided in to two equal groups (n = 6/group) and, fasted and hydrated as described previously. One group was orally administered with 2 ml of water and the other with 167 mg/ml of BTB. These rats were then individually placed in metabolic cages and their cumulative urine output measured for 6 h. The urine samples were then subjected to the following investigations: Na^+, K^+, Ca^{++} and Mg^{++} levels with a flame photometer (Compact Atomic Absorption Spectrometer, GFS Scientific Equipment PVT. Ltd., Sydney, Australia). Cl^- level was assayed by titrometrically with $AgNO_3$ using phenolpthaline, osmolarity with an osmometer (V/ESCOR 5500 vapor pressure osmometer). pH, specific gravity, glucose, bilirubin, urobilinogen, ketone, nitrite, traces of blood, and leucocytes were determined using Uritest 10C urine reagent strips (Guilin Medical Electronic Instrument Factory, Guiline, China).

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 administed with 2 ml of water and the other with 167 mg/ml of BTB. These rats were then individually placed in metabolic cages and their cumulative urine output was measured after 2h and 24h. Blood was also collected from tail using aseptic precautions and serum was separated. Creatinine levels in the serum and urine were determined using Randox kits (Randox laboratories Ltd., Antrim, UK). Creatinine clearance was computed as per instructions given by the manufacturer using the above data creatinine clearance was taken as an estimation of the glomerular filtration rate (14).

Evaluation of acute and chronic toxicity

Twelve male rats were randomly assigned into two equal groups (n = 6/group). The first group was orally treated for 62 consecutive days (at 9.00 h) with the 167 mg/ml of BTB and the other with 2 ml of water. During this period, each rat was observed for overt sings of toxicity (salivation, lachrymation, breathing distress, ptosis, stupor, squint, teeth exposure, writhing, convulsions, tremors, yellowing of fur, loss of fur), stress (erection of fur and exophthalmia), behavioural abnormalities (such as impariment of spontaneous movements, climbing, cleaning of face and ataxia, rolling and other postural changes) and aversive behaviour (biting and scratching, licking of tail, paw and penis, intense grooming or vocalization) and diarrhoea. On day 1 post treatment (63^{rd} day), these rats were anaesthetised with ether and blood was collected from tails using aseptic precautions. Estimations of serum urea, creatinine, Na^+ and K^+ (to examine renal toxicity), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (to examine liver toxicity) were made using respective assay kits (Randox Laboratories Ltd., Antrim, UK). The serum Na^+ and K^+ levels were estimated using a flamephotometer.

Statistical analysis

Data are given as means ± standard error of the mean (SEM). Statistical comparisons were made using Mann-Whitney U- test. Significant level was set at $P < 0.05$.

RESULTS

Evaluation of diuretic activity

There was no significant difference ($P > 0.05$) in the cumulative urine output of male and female rats subjected to different treatments (data not shown). Therefore, the results were pooled wherever relevant.

As shown in Table 1, mid dose (by 36%), high dose (by 47%), supraphysiological dose (by 51%) of BTB and the reference drug frusemide (by 65%) significantly increased ($P < 0.05$) the cumulative urine output. The diuretic effect induced by BTB was curvilinearly dose-dependent (r^2=0.98, $P < 0.05$). EC_{50} for the diuretic effect was 370 mg/ ml. The onset of diuresis with BTB was evident at 2 h (mid dose by 95 %, high dose by 142 %, and supraphysiological dose by 110 %) and the peak diuretic effect at 3 h (mid dose by 100 %, high dose by 150 % and supraphysiological dose by 144 %) (see Figure 1). The diuretic effect subsided thereafter to non-significant levels ($P > 0.05$).

On the other hand, with frusemide a significant enhancement ($P < 0.05$) of urine output was seen only at 1h (by 88%) and 2h (by 72%) with the peak effect at 1h (Figure 1). The mid dose of BTB made with decaffeinated Dust tea failed (only by 0.5%) to induce significant ($P > 0.05$) diuresis. With subchronic administration of mid dose of BTB there was a significant increase ($p<0.05$) in urine output on both day 14 (control vs treatment: 1.9 ± 0.12 vs 3.7 ± 0.44 ml) and day 28 (2.1 ± 0.15 vs 3.7 ± 0.25 ml). However, the cumulative urine output of treated rats on these two days were not significantly different ($P > 0.05$).

Evaluation of urinary frequency

The results show that mid dose (167mg/ml) of Dust tea brew significantly ($P < 0.05$) increased the frequency of urination at 1^{st} h by 31% (control vs treated: 3.67 ± 0.17 vs 5.33 ± 0.22) and 2^{nd} h by 30% (3.5 ± 0.13 vs 5.00 ± 0.4) of post treatment, and the total number of

urinations by 26% (10.33 ± 0.5 vs 14.00 ± 0.91) during the entire 6 h period.

Urine analysis

As shown in Table 2, the mid dose (167 mg/ml) of BTB slightly (by 11%) but significantly ($P < 0.05$) increased the urinary pH. This dose also provoked a 55% increase in urinary Na^+ excretion ($P < 0.05$). However, the urinary Na^+/K^+ ratio remained unaltered. On the other hand, the remaining parameters determined were either not significantly changed ($P > 0.05$) (K^+, Ca^{++}, Mg^{++}, Cl^- and osmolarity) or not detected in the urine samples. Further, the colour of the urine of treated rats seemed almost identical to that of vehicle treated rats (controls).

Evaluation of creatinine clearance

The mid dose of BTB caused a marked (813%) and significant ($p< 0.05$) enhancement in glomerular filtration rate, as estimated by creatinine clearance at 2h post treatment. (Control vs treatment: 0.031 ± 0.009 vs 0.304 ± 0.08 ml/min)

Evaluation of chronic toxicity

The mid dose of BTB did not provoke any overt signs of toxicity, stress, behavioral abnormalities or aversive behaviours. Additionally, none of the serum parameters investigated was significantly altered ($P > 0.05$) by the mid dose of BTB (urea: control vs treatment: 19.64 ± 0.16 vs 9.99 ± 0.60 mg/dl, creatinine: 1.07 ± 0.16 vs 0.43 ± 0.04 mg/dl, SGOT: 26.66 ± 0.55 vs 23.25 ± 2.25 U/l, SGPT: 10.32 ± 0.20 vs 8.35 ± 0.13 U/l, Na^+: 7078.23 ± 128.50 vs 7835.20 ± 98.50 ppm, and K^+: 285.23 ± 5.23 vs 359.24 ± 3.86 ppm).

DISCUSSION

This study examined the oral diuretic potential of BTB of *C. sinensis* made from Sri Lankan high grown Dust grade No: 1 black tea (which is pure, unblended and typical to the grade) in rats. In contrast to what has been reported by Goonaratna et al. (4) the results of this study showed that BTB made from *C. sinensis* of Sri Lanka possesses mild to moderate oral diuretic activity, justifying the claim made by Sri Lankan traditional practitionars that it increases the urinary output. The lack of diuretic activity of BTB of *C. sinensis* evident in the study of Goonaratna et al. (4)

Table 1: Effect of oral administration of Sri Lankan black tea of Camellia sinensis on cumulative urine output of rats (mean ±SEM)

Treatment	Dose	Mean cumulative urine output (ml) over 6h
Control	2ml water (n = 27)	2.14±0.05
Dust grade No1		
	84 mg/ml (low) (n = 19)	2.80±0.10
	167 mg/ml (mid) (n = 18)	3.37±0.06*
	501 mg/ml (high) (n = 25)	4.06±0.12*
	1336 mg/ml (Supraphysiological) (n = 21)	4.40±0.12*
Decaffeinated Dust No 1	167 mg/ml (mid) (n = 9)	2.15±0.03
Frusemide	13mg/Kg (n = 9)	6.1±0.12*

*As compared to control *P < 0.05*

Table 2: Effect of orally administered Sri Lankan BTB of Camellia sinensis on some urine parameters of rat (mean ±SEM)

Parameter	Control group (n = 12) (2 ml water)	Treated group (n = 12) (167 mg/ml of BTB)
pH	6.66±0.37	7.5±0.91*
H^+ (ppm)	$2.18x10^{-4} \pm 0.001$	$3.16x10^{-5} \pm 0.004$*
Specific gravity	1.019±0.005	1.017±0.003
Cl^- (ppm)	9287.8±2930	9914.8±3351
Na^+ (ppm)	3656.6±802.9	5680.9±1876.4*
K^+ (ppm)	2081.4±1235	2572.8±1145
Na^+/K^+ ratio	2.23±0.89	2.42±0.85
Ca^{++} (ppm)	445.1±105.7	495.1±90
Mg^{++} (ppm)	222.6±58.3	197.6±45
Osmolarity (m.mol/kg)	879.61±280.7	923.8±385.7
Glucose	ND	ND
Bilirubin	ND	ND
Ketones	ND	ND
Blood	ND	ND
Urobilinogen	ND	ND
Nitrite	ND	ND
Leucocytes	ND	ND

*As compared to control *P < 0.05; ND= Not detected; BTB = black tea brew*

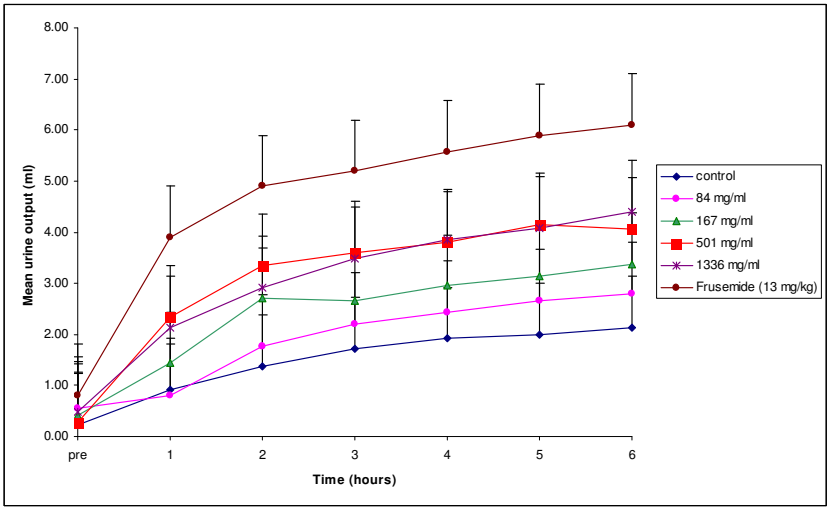

Figure 1. Cumulative urine output of rats orally administered with Sri Lankan black tea brew of Camellia sinensis (mean ± SEM) ** p < 0.05, compared with the control (Mann-Whitney U test)*

may be due to usage of lower volume of weak aqueous infusion of tea. The diuretic activity of BTB was dose-dependent indicating that this effect is genuine, intrinsic, causal and possibly receptor mediated (15, 16). However, the therapeutic efficacy of BTB was about 45% less than frusemide, a widely used diuretic in allopathic medicine (16). The diuretic activity of BTB had a fairly rapid onset (within 2 h) and relatively short duration of action (upto 3 h) indicating quick absorption of active constituent/s and equally rapid metabolism and/or clearance. Such an action profile is useful in some forms of diuretic therapy.

The frequency of urination during the study period was markedly increased possibly indicating depressor instability and/or an effect on micturition reflex (15). Further, the diuretic response observed was not gender dependent. In contrast, *in vivo* antioxident activity of BTB of *C. sinensis* in humans is shown to be gender dependent (17). BTB induced diuresis remained undiminished even with chronic administration in this study indicating lack of development of tolerance. This is an important feature expected of any drug.

Plant extracts with high salt content is shown to produce diuresis (18). But such a nonspecific mechanism is unlikely to be operative here as BTB has been shown to have low salt content (19). Some herbal drugs induce diuresis by stimulating the thirst center in the hypothalamus and thereby enhancing the fluid intake (18). Even this mode of action is unlikely in this study as the rats had no access to water after the BTB was administered. It is well recognized that ADH plays a vital role in the regulation of urinary output (15, 16). A possibility exist that the black tea may have stimulated diuresis by impairing the pituitary release of ADH as is reported with ethyl alcohol or water (15) and/or by impairing the responsiveness of ADH to water resorption at the uriniferous tubules (20). But, even this mode of action is unlikely to be operative here as there was no change in the specific gravity and osmolarity of urine following BTB administration. Further, inhibition of ADH causes polyurea with low osmolarity (20).

The diuresis induced by BTB can be therefore solely be attributed to marked increase (by 55 %) of urinary Na^+ loss: all the therapeutically used diuretics are known to increase urinary Na^+ excretion (15, 21). Phytochemical analysis of the tea sample has shown the presence of caffeine, a methlylxanthine (6, 19, 22). All methlylxanthines have a mild diuretic effect (15, 22). The diuretic action of the BTB can be mainly, if not solely, attributed to caffeine since decaffeination almost completely abolished its diuresis.

One mechanism by which BTB can elevate urinary Na^+ level is by inhibiting the Na^+ resorption in the nephron. This mode of action is likely to be operative here as caffeine which is reported to be present (19) in the black tea sample is known to inhibit Na^+ resorption at nephrons (15, 23). BTB induced a marked increase in glomerular filtration rate, which may be yet another way to promote urinary Na^+ excretion (15, 23) by the BTB. Such a mode of action can be operative with BTB as caffeine has been shown to increase renal blood flow (15, 23) and cardiac output (15, 23).

Interestingly, no overt signs of toxicity, hepatotoxicity (in terms of SGOT and SGPT) or renotoxicity (in terms of serum urea, creatinine, Na^+ and K^+ levels) were evident even following chronic administration of the tea brew.

In conclusion, this study shows for the first time that BTB of *C. sinensis* made from Sri Lankan high grown Dust grade tea possesses safe, mild to moderate oral diuretic activity justifying the claims made by Sri Lankan traditional practitionars.

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