

Antipyretic activity of Sri Lankan black tea (*Camellia sinensis*)

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ABSTRACT

This study examined the antipyretic activity of Sri Lankan black tea (*Camellia sinensis* L.) in yeast-induced rat pyrexia model using black tea brew (BTB) of high grown Dust grade No: 1 tea. Different doses of BTB (167 mg/ml, equivalent to 3 cups; 501 mg/ml, equivalent to 9 cups; and 1336 mg/ml, equivalent to 24 cups), water (control) or paracetamol (200 mg/kg, reference drug) were orally administered to yeast -induced pyretic rats (N = 6/group) and their rectal temperature monitored at hourly intervals for 6 h. The results show that all doses of BTB and paracetamol significantly ($P < 0.05$) suppressed the pyrexia-induced by yeast (low dose upto 2 h, mid dose upto 5 h, high dose upto 4 h and paracetamol upto 4 h). In addition, the mid dose of BTB significantly ($P < 0.05$) suppressed the intestinal secretion in enteropooling assay of mice suggesting an impairment of prostaglandin synthesis. It is concluded that Sri Lankan black tea possesses antipyretic activity of moderately long duration in rats and it could play a similar role in humans.

Key words: *Camellia sinensis*; black tea; pyrexia; antipyretic

INTRODUCTION

Tea which is manufactured from the topmost immature leaves and the bud of *Camellia sinensis* (L) O. Kuntze (Family Theaceae) plant is one of the most popular beverages consumed worldwide. Depending on the manufacturing process there are three main types of teas: black (fully aerated or fermented) green (unaerated or unfermented) and oolong (partially aerated or semifermented) (Modder and Amarakoon, 2002). It is estimated that 80% of world produced tea is consumed as black tea (Anonymous, 2004) predominantly in North America, Great Britain and some Asian countries (Kunkel, 2003).

Tea and health have always been inextricably linked. Several laboratory studies have demonstrated that tea, especially the green tea, exhibit distinct and diverse pharmacological properties: antioxidative; anti-inflammatory; anticarcinogenic; antimutagenic; antiangiogenic; antiarteriosclerotic; antiobesity; antidiabetic; antiageing; hypocholesterolaemic; antibacterial; antiviral; impairment of digestive enzyme activity; alleviation of liver ailments and neurological conditions or reduction of tooth decay and other gum ailments (Modder and Amarakoon, 2002; Koo and Cho, 2004). Furthermore, findings from several epidemiological studies suggest that regular consumption of moderate to high quantity of tea lower the risk of heart diseases, stroke and cancers (Modder and Amarakoon, 2002; Koo and Cho, 2004).

Sadly, less attention has been focused on the bioactivity of black tea which is heavily consumed by the tea drinkers in the world. In this regard, it has been suggested that more effort should be focused on black tea research in the future (Chi-Tang Ho *et al.*, 2005). With respect to Sri Lankan unblend garden mark black tea (the second largest producer and exporter) published studies on its bioactivity are extremely limited (Abeywickrama, *et al.*, 2004; Abeywickrama, *et al.*, 2005; O'Mahony, *et al.*, 2005; Ratnasooriya and Amarakoon, 2007; Ratnasooriya, *et al.*, 2007).

Therefore, we have initiated a research programme to investigate bioactivities of Sri Lankan black tea. In this study, we report antipyretic activity of Sri Lankan black tea using high grown Dust grade No: 1 tea in rats.

MATERIALS AND METHODS

Animals

Laboratory bred healthy adult male Wistar rats (weighing 200-225 g) and ICR strain male mice (weighing 30-40 g) purchased from Medical Research Institute, Borella, Sri Lanka were used. They were kept under standardized animal house conditions (temperature: 28-31 °C, photoperiod: approximately 12 hours of light per day, relative humidity 50-55%). They had free access to pelleted food (Ceylon Grain Elevators, Colombo, Sri Lanka) and tap water. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care (based on Helsinki convention) and guidelines and rules of the Faculty of Science, University of Colombo, for animal experimentation.

Source of tea

Two or three topmost immature leaves and buds of *C. sinensis* plants 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 was used to process Dust grade No: 1 black tea by orthodox-rotovane 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 °C until use.

Preparation of black tea brew (BTB)

BTB was made according to ISO standards (Anonymous, 1980): adding 2 g of black tea to 100 ml water and brewing for 5 min [yield (w/w) 43.7%]. For oral administration of rats, 3 concentrations of BTB (167 mg/ml, equivalent to 3 cups; 501 mg/ml, equivalent to 9 cups and 1336 mg/ml, equivalent to 24 cups) were made in 2 ml of water. The volume of 1 cup is considered as 170 ml.

Effects on rectal temperature

Thirty rats were randomly assigned into 5 equal groups (N = 6/group). The rectal temperature of these rats were determined using a digital thermometer (CT 461C, Citizen systems Japan Co., LTD, Tokyo, Japan). These rats were then subcutaneously injected with 10 ml/kg of 15% (W/V) aqueous suspension of active dry yeast (General Milling Corporation, Lapu-Lapu City, Philippines) to induce pyrexia. Nineteen hours after injection of yeast, rectal temperature of these rats was determined (Hullatti and Sharada, 2007). These rats were then orally treated with BTB, water or paracetamol as follows: group 1, 2 ml of distilled water, group 2 with 167 mg/ml of BTB, group 3 with 501 mg/ml of BTB, group 4 with 1336 mg/ml of BTB and group 5 with 200 mg/kg of paracetamol (reference drug). Rectal temperature was then determined at 1, 2, 3, 4, and 5 h post treatment.

Effect of BTB on small intestinal secretion

Intestinal secretion was indirectly evaluated by the enteropooling assay (Vitali *et al.*, 2005). Briefly, 18 mice were randomly divided into three groups (N = 6/group). Mice in group 1 were orally treated with 0.2 ml of water, group 2 with 0.2 ml water and group 3 with 501 mg/ml of BTB. Forty minuits later, mice in groups 2 and 3 were orally administered with 0.2 ml of castor oil. After 30 min all the mice were sacrificed using ether and their small intestines were removed and weighed. The weights were then expressed as mg/20g body weight. The difference in the intestinal weight between the normal control and castor oil treated control was considered as the castor oil-induced accumulation of intestinal fluid.

Statistical analysis

Data are given as means \pm SEM. Statistical comparisons were made using the Mann-Whitney U-test. $P \leq 0.05$ was considered as significant.

RESULTS

The results obtained with rectal temperature measurements are summarized in Table 1. As shown, subcutaneous administration of yeast induced pyrexia in rats ($> 100^{\circ}\text{F}$). BTB 167 mg/ml dose significantly ($P < 0.05$) suppressed the pyrexia only at 1st and 2nd h post-treatment. On the other hand, 501 mg/ml and 1336 mg/ml doses of BTB significantly ($P < 0.05$) reduced pyrexia up to 5 h and 6 h respectively. The reference drug paracetamol also significantly ($P < 0.05$) reduced pyrexia up to 4 h. The antipyretic potential of black tea brew was essentially comparable to that of paracetamol (200 mg/kg).

The results in the enteropooling assay are summarized in Table 2. As shown, oral administration of castor oil significantly ($P < 0.05$) increased the intestinal fluid secretion, compared with the normal control. BTB on the other hand, significantly ($P < 0.05$) inhibited the castor oil-induced intestinal secretion.

Table 1. Effects of oral administration of black tea brew (BTB) of Dust grade No: 1 tea (167, 501, and 1336 mg/ml) and paracetamol on yeast-induced pyrexia in rats (mean \pm SEM)

Treatment	Temperature °F						
	Before yeast	After yeast	Post treatment				
			1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour
Control (Water)	98.5 \pm 0.30	101.15 \pm 0.29	101.13 \pm 0.24	101.32 \pm 0.16	101.00 \pm 0.34	101.02 \pm 0.28	100.58 \pm 0.25
BTB							
167 mg/ml (eq. 3 cups)	98.3 \pm 0.32	101.02 \pm 0.15	99.55 \pm 0.23*	99.76 \pm 0.02*	100.07 \pm 0.07	100.17 \pm 0.09	100.07 \pm 0.04
501 mg/ml (eq. 9 cups)	98.9 \pm 0.38	100.20 \pm 0.03	99.54 \pm 0.15*	99.13 \pm 0.15*	99.56 \pm 0.14*	99.67 \pm 0.18*	99.16 \pm 0.25*
1336 mg/ml (eq. 24 cups)	98.3 \pm 0.40	101.70 \pm 0.12	99.63 \pm 0.16*	99.46 \pm 0.15*	99.40 \pm 0.19*	99.45 \pm 0.14*	99.56 \pm 0.12
Paracetamol (200 mg/kg)	98.9 \pm 0.43	100.98 \pm 0.23	98.66 \pm 0.09*	98.73 \pm 0.06*	98.83 \pm 0.03*	99.08 \pm 0.03*	99.18 \pm 0.07

* = P < 0.05 compared to control (Mann-Whitney U-Test); eq = equivalent

Table 2. Effect of oral administration of 501 mg/ml black tea brew (BTB) of Dust grade No: 1 tea on castor oil-induced enteropooling in mice (mean \pm SEM)

Treatment	Small intestine weight (mg/20g)	Castor oil-induced intestinal fluid accumulation (mg)
Normal control (water)	829.4 \pm 2.3	-
Castor oil control (0.2 ml castor oil + water)	1337.2 \pm 2.8 ^a	507.8
501 mg/ml of BTB (0.2 ml castor oil + 501 mg/ml BTB)	1029.3 \pm 3.5 ^{ab}	199.9

^a P < 0.05 compared to normal control , ^b P < 0.05 compared to castor oil control (Mann-Whitney U-Test)

DISCUSSION

The ancients believed that green tea is beneficial for regulating body temperature (Anonymous, 2005) and might be useful in malaria (Stagg and Millin, 1975). On the other hand, antipyretic effects of black tea is not mentioned either in Ayurvedha or folklore. This study shows, for the first time, that black tea made from Sri Lankan high grown Dust grade No: 1 tea possesses significant antipyretic activity when tested in yeast-induced pyrexia model of rats. This model is validated, reproducible, reliable and widely used to test antipyretic effects of potential drugs (Gupta *et al.*, 2005; Hullatti and Sharada, 2007). Therefore, the results obtained are genuine and reassuring. This is an important finding with clinical significance.

The antipyretic effect of BTB was prompt (appeared within 1h) and moderately long (lasted up to 4-5h) like the reference drug paracetamol which is frequently used therapeutically to alleviate pyrexia (Rang *et al.*, 1995). Further, in the rat model BTB had a fever suppressing effect similar to paracetamol (with respect to magnitude and duration).

Sweating reduces body temperature (Carola *et al.*, 1990). But this antipyretic effect is not mediated via sweating as rats do not have sweat glands (Matthews, 1970). Tea contains appreciable amount of caffeine (Modder and Amarakoon, 2002). Caffeine is a known vasoconstrictor (Benowitz, 1990). Hence, constriction of skin blood vessels would be expected. Thus, reduction of body temperature due to radiation is unlikely. The thermoregulatory center in the hypothalamus controls the set body temperature of mammals (Carola *et al.*, 1990). Prostaglandins are known to elevate the thermoregulatory set point and thereby produce pyrexia (Carola *et al.*, 1990). Prostaglandin synthesis blockers cyclooxygenase inhibitors (cox) like aspirin, paracetamol, ibuprofen (Rang *et al.*, 1995) reduce elevated temperature in pyrexia. Therefore, it is possible that BTB may also suppress

yeast-induced pyrexia by a similar mechanism. Indeed, inhibition of expression of cox-2 has been reported in black tea extracts (Luceri *et al.*, 2002). Furthermore, reduction of intestinal weight in the castor oil experiment suggests an inhibition of prostaglandin biosynthesis (Gunakkanru *et al.*, 2005). There are other mediators such as cytokines, tumor necrosis factor (Rang *et al.*, 1995) underlying pathogenesis of fever. Possibility exists that BTB could also inhibit these mediators in bringing about antipyresis. Further studies are obviously needed to clarify this point.

In conclusion, this study, shows for the first time, that Sri Lanka black tea possesses marked antipyretic activity in rats. Further, research are necessary to demonstrate similar effects in human.

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