

Effect of a high dose of Sri Lankan black tea brew (*Camellia sinensis*) on body weight, liver and kidney functions in rats

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Abstract

*The aim of this study was to examine general, hepatic and renal toxicities with chronic daily administration of Sri Lankan black tea (*Camellia sinensis* L., Family Theaceae). This was tested in rats using black tea brew (BTB) made from Sri Lankan high grown Dust grade No: 1 black tea. Either a single heavy dose of BTB (501 mg/ml, equivalent to 9 cups) or water (control) were orally administered to two groups of rats ($n = 9$ /group) daily for 8 weeks. These rats were observed daily for overt signs of clinical toxicity. Blood samples were collected at two weeks intervals and levels of serum proteins, GOT, GPT, creatinine, urea, Na^+ , K^+ , and Na^+/K^+ ratio were determined. Body weights of these rats were determined fortnightly and food intake were noted. The results show that BTB administration did not induce any overt signs of toxicity or produced significant ($p > 0.05$) change in any of the serum parameters investigated. On the other hand, a significant ($p < 0.05$) weight loss (6.6 - 10.4%) was evident in BTB treated rats after 2 weeks. It is concluded that chronic heavy consumption of Sri Lankan black tea appears to be non toxic to liver and kidney but it suppresses the body weight of a rats.*

Short running head: Sri Lankan black tea and toxicity

Key words: *Camellia sinensis*, liver toxicity, renal toxicity, body weight, Sri Lankan tea, black tea

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1. Introduction

Most people enjoy tea on a daily basis. Tea is the second most consumed beverage in the world at present. It is estimated that globally 3-5 billion cups, glasses or bowls of tea consumed daily at present. Tea is typically produced from freshly harvested tender shoots comprising two or three of topmost immature leaves and the bud of *Camellia sinensis* L. plant (Family: Theaceae) [1]. Depending on the manufacturing technique there are three main types of teas; black (fully aerated or fermented), green (non aerated or unfermented) and oolong (partially aerated or semifermented).

Of these types, black tea accounts for about 78% of world tea production and about 80% of global tea consumption. It is of interest to note that Sri Lankan tea is drunk in more than 125 countries and accounts for 20% of the global tea consumption [2]. Although there is a high consumption of Sri Lankan black tea no studies are conducted to investigate its toxic effects with long term consumption. Black tea contains a large variety of chemicals constituents such as flavonoids (catechins, theaflavins, thearubigins, and flavonols), caffeine, amino acids, volatiles, vitamins and minerals [1, 3]. In addition, it may also be contaminated with pesticidal and weedicidal residues. Therefore, a possibility exists that heavy daily consumption of black tea brew (BTB) chronically could produce toxic effects.

The aim of this study was to examine general, hepatic and renal toxic effects of Sri Lankan black tea with a heavy chronic daily administration. This was tested in rats using high grown Dust grade No: 1 Sri Lankan black tea. The dust grade was selected is the most widely consumed type of tea by Sri Lankan tea drinkers.

2. Materials and Methods

2.1. Animals

Laboratory bred healthy adult male Wistar rats (weighing 275-300 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 (contents; 19.5% proteins, 7.5% oil, 4.5% fiber, 7.9% ash, 0.48% methylamine, 0.9% calcium and 0.7% phosphorus) (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.

2.2. 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.

2.3. Preparation of black tea brew (BTB)

BTB was made according to ISO standards [4]: adding 2g of black tea to 100ml water and brewing for 5 min. This contains 43.7% (w/w) tea solids in water [5]. Based on this data, a dose of 501 mg/ml of BTB in 2 ml was made by adding 8g black tea to 20 ml boiling water and brewing for 5 min. In human terms this concentration is equivalent to 9 cups of tea (1 cup = 170 ml).

2.4. Investigation of toxic effects following chronic administration

Eighteen rats were randomly divided into two groups (n = 9/group) and were caged individually. One group was orally administered with 501 mg/ml of BTB and the other with 2 ml of water daily at (9.00- 10.00 h) for 8 weeks. These rats were observed daily (2-3 h following administration) for overt signs of toxicity (diarrhoea, jumping, restlessness, salivation, rhinorrhoea, lachrymation, chewing jaw movements, potosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair, ataxia, rapid rotational movement of head, neck and entire body around the spinal axis, pallor of lips, marked impairments of food and water intake, lethargy and sleepiness), stress (fur erection and exophthalmia), behavioural abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, rolling and other postural changes) and aversive behaviours (biting and scratching behaviour, licking at tail, paw and penis, intense grooming behaviour or vocalisation).

Every two weeks during the study period, blood (1.5 – 2.0 ml) was collected from the tails of these rats under mild ether anaesthesia using aseptic precautions. The blood was allowed to clot at room temperature (28 – 30 °C) and centrifuged at 3200 rpm for 5 min. The serum was separated. Serum protein, Glutamic-Oxaloactic Transaminase (GOT) (EC 2.6.1.1), Glutamic-Pyruvic Transaminase (GPT) (EC 2.6.1.2), creatinine and urea levels were

determined using Randox kits (Randox Laboratories Ltd., Co., Antrium, UK) and a spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan) as per manufactures instruction (wave length for SGOT, SGPT, protein and urea -546 nm; for creatinine- 492 nm). Serum Na⁺ and K⁺ levels were determined by flame photometrically (Compact atomic absorption spectrometer, GFS Scientific Equipment Pvt. Ltd., Sydney, Australia). The body weights of these rats were also determined 15- 30 min before blood collection using an electronic balance (MP 6000, Chyo Balance Corp, Tokyo, Japan).

2.5. Statistical analysis

The data are expressed as mean \pm standard error of mean (SEM). Statistical comparisons were made using Mann-Whitney U-test. A probability level of 0.05 or less was considered as significant.

3. Results

Chronic administration (8 weeks) of 501 mg/ml of BTB did not induce any overt signs of toxicity, stress or aversive behaviors. Further, as shown in Table 1, the BTB treatment also did not significantly ($p > 0.05$) change the serum proteins, GOT, GPT, creatinine, urea, Na⁺, K⁺ levels and serum Na⁺/K⁺ ratio. In contrast, BTB treatment caused a significant ($p < 0.05$) impairment in the body weight (Figure 1) from the second week (6.6 - 10.4%) although there was no apparent reduction in food intake. This effect on body weight was time-dependent ($r^2 = - 0.87$; $p < 0.05$). However, the animals were active and did not appear lethargic and morbid. Further, no mortality was evident during the study period.

4. Discussion

This study examined the general toxicity (in terms of overt clinical signs), hepatotoxicity (in terms of SGOT, SGPT and serum proteins) and renotoxicity (in terms of serum creatinine, urea, Na⁺, K⁺ and Na⁺/K⁺ ratio) following chronic (8 weeks) administration of a heavy dose of BTB made from Sri Lankan high grown Dust grade N9: 1 black tea. The dose selected was 501 mg/ml/day which is equivalent to 9 cups/day. In Sri Lanka, drinking 3 cups of tea a day is usually considered as normal and consumption of 10 cups per day is generally regarded as heavy [1]. The results show that chronic administration of a high dose of Sri Lankan BTB dose not induce general toxicity, renotoxicity or hepatotoxicity in rats. A similar result may be expected in humans but this needs further investigations with human subject; five cases of liver toxicity have been reported recently with consumption of a tea

beverages [6]. This is a novel and an encouraging finding for Sri Lankan black tea as it accounts for 20% of the global tea consumption [2]. On the other hand, BTB induced a quick reduction in body weight. Further, this reduction in body weight was not drastic and progressed gradually. This is also an important finding which could have a clinical benefit, especially, in obese states. Black, oolong [5] and green tea [8] are claimed to be antiobesetic and promote weight loss [7, 9, 10]. The weight loss in this study was unlikely to be due to suppression of food intake as there was no apparent reduction in feeding. However, epigallocatechin but not related catechins of green tea is reported to reduce food intake of rats [11]. Further, the BTB treated rats were not lethargic and morbid and therefore the reduction in weight may not be due to toxicity. In contrast, BTB induced weight reduction could be mediated via raising of metabolic rates and promotion of fat oxidation [10, 12]. Such an action can be mediated [10] by caffeine and other flavonoids present in tea [1, 3]. Drinking of green tea has been shown to lower body weight by increasing thermogenesis [10, 13]. Such a mode of action is possible in this study as well.

In conclusion, this study shows that Sri Lankan black tea dose not induce overt signs of toxicity, renal toxicity or hepatic toxicity when consumed daily at a high dose level for a long period (upto 8 weeks) in rats. However, the treatment produced a weight loss, which is an encouraging finding of clinical importance.

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Table 1: Effect of chronic oral administration of 501 mg/ml of black tea brew of *Camellia sinensis* on some serum parameters of rats (mean \pm SEM; n = 9)

Parameter	Dose	Pre treatment	During treatment			
			2 Weeks	4 Weeks	6 Weeks	8 Weeks
Total proteins (mg/ml)	Control (water)	6.83 \pm 0.008	6.85 \pm 0.008	6.86 \pm 0.005	6.87 \pm 0.001	6.91 \pm 0.001
	BTB (501 mg/ml)	6.82 \pm 0.002	6.88 \pm 0.006	6.87 \pm 0.004	6.77 \pm 0.003	6.86 \pm 0.002
Serum GOT (U/L)	Control (water)	33.4 \pm 0.02	33.8 \pm 0.02	35.0 \pm 0.02	36.2 \pm 0.07	38.2 \pm 0.10
	BTB (501 mg/ml)	33.5 \pm 0.02	34.2 \pm 0.04	35.5 \pm 0.05	36.4 \pm 0.07	38.9 \pm 0.06
Serum GPT (U/L)	Control (water)	10.65 \pm 0.03	10.61 \pm 0.06	11.35 \pm 0.02	12.00 \pm 0.02	12.60 \pm 0.06
	BTB (501 mg/ml)	10.98 \pm 0.05	10.96 \pm 0.05	11.24 \pm 0.05	11.21 \pm 0.03	12.5 \pm 0.01
Urea (mg/ml)	Control (water)	31.6 \pm 0.11	31.8 \pm 0.11	32.9 \pm 0.09	33.1 \pm 0.15	33.5 \pm 0.15
	BTB (501 mg/ml)	32.3 \pm 0.70	32.3 \pm 0.70	32.8 \pm 0.05	34.5 \pm 0.14	34.0 \pm 0.20
Creatinine (mg/ml)	Control (water)	0.73 \pm 0.002	0.75 \pm 0.002	0.78 \pm 0.03	0.81 \pm 0.002	0.83 \pm 0.001
	BTB (501 mg/ml)	0.73 \pm 0.003	0.75 \pm 0.001	0.78 \pm 0.003	0.82 \pm 0.002	0.85 \pm 0.003
Na ⁺ (ppm)	Control (water)	7086.8 \pm 1.7	7083.7 \pm 3.5	7084.9 \pm 0.8	7081.4 \pm 4.6	7084.9 \pm 4.2
	BTB (501 mg/ml)	7082.8 \pm 4.8	7084.2 \pm 2.3	7084.0 \pm 3.3	7082.1 \pm 3.7	7088.9 \pm 2.0
K ⁺ (ppm)	Control (water)	286.89 \pm 0.75	286.11 \pm 0.48	286.33 \pm 0.53	285.67 \pm 0.90	286.56 \pm 0.56
	BTB (501 mg/ml)	285.67 \pm 1.47	285.89 \pm 0.70	287.00 \pm 0.58	286.22 \pm 0.62	285.22 \pm 0.64
Na ⁺ /K ⁺ ratio	Control (water)	24.70 \pm 0.09	24.75 \pm 0.1	24.75 \pm 0.01	24.78 \pm 0.08	24.72 \pm 0.1
	BTB (501 mg/ml)	24.72 \pm 0.08	24.78 \pm 0.2	24.77 \pm 0.3	24.76 \pm 0.02	24.74 \pm 0.2

* P < 0.05 compared to control (Mann-Whitney U- test); GOT = Glutamic-Oxaloacetic Transaminase, GTP = Glutamic-Pyruvic Transaminase

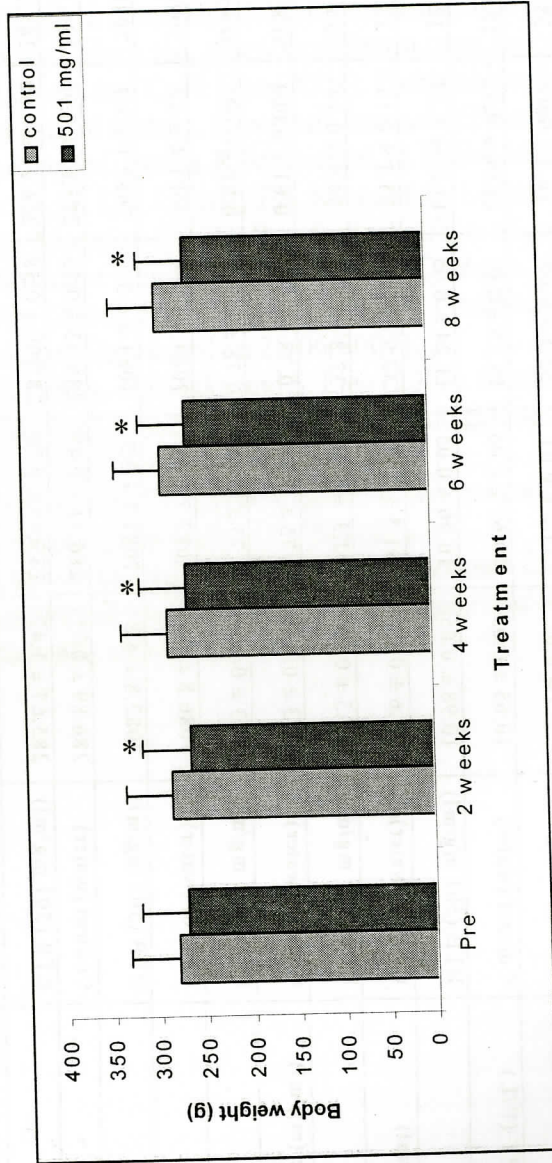


Figure 1: Body weight of rats orally administered with Sri Lankan black tea brew of *Camellia sinensis* (mean \pm SEM)
* p < 0.05, compared with the control (Mann-Whitney U test)