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Effect of black tea brew of *Camellia sinensis* on oestrous cycle of rats

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ABSTRACT

This study examined the effects of black tea brew (BTB) of *Camellia sinensis* (L.) O. Kuntze (Family: Theaceae) on oestrous cycle using Sri Lankan high grown Dust grade No: 1 black tea and rats. Different doses of BTB (84 mg/ml, equivalent to 1.5 cups, 168 mg/ml, equivalent to 3 cups and 501 mg/ml, equivalent to 9 cups) or 2 ml of water (control) were orally administered for 31 consecutive days and the oestrous cycle was monitored daily by vaginal smearing. The results showed that none of the doses of BTB significantly ($P > 0.05$) altered the percentage of occurrence of different stages of the oestrous cycle and its duration. It is concluded that daily chronic administration of even high dose of BTB (equivalent to 9 cups) dose not disrupt the oestrous cycle of rats.

Key words: *Camellia sinensis*; black tea; oestrous cycle; menstrual cycle

INTRODUCTION

Tea which is manufactured from the topmost immature leaves and the bud of *Camellia sinensis* (L.) O. Kuntze (Family Theaceae) plant is currently the second most consumed beverage of the world (Modder and Amarakoon, 2002). Based on the method of manufacturing process there are three main types of teas: black (fully aerated or fermented) green (unaerated or unfermented) and oolong (partially aerated or semi fermented). Of these, black tea accounts for about 78% of the global tea consumption (Anonymous, 2004).

A typically black tea brew (BTB) contains 0.35% tea solids in water (Modder and Amarakoon, 2002). Upto 40 % of the solids extracted to water are flavonoids (catechins, theaflavins, thearubigins, flavonols) and infact tea is one of the richest sources of natural flavonoids (Modder and Amarakoon, 2002). BTB also contains appreciable amounts of alkaloid, caffeine and amino acid, theanine (Balentine *et al.*, 1997; Modder and Amarakoon, 2002).Generally, coffee and tea are the main sources of caffeine for adults (Fenster *et al.*, 1999). Most of the known bioactivities of black tea are linked with these three phytochemical constituents (Modder and Amarakoon, 2002).

Consumption of caffeine is known to induce disturbances in the menstrual cycle in women (Fenster et al., 1999): shortens the duration of menstrual cycle and menstruation. Further,

caffeine increases uterine vascular resistance and reduce uterine blood flow (Wilson *et al.*, 1983) and affects serum LH and FSH levels (Polan *et al.*, 1983) which could intern affect the menstrual cycle length. The mechanism of caffeine induced disturbances in the menstrual cycle is not clear but likely to be due to its effects on sex hormones or their receptors (Fenster *et al.*, 1999) as sex hormones initiate and regulate oestrous cycle of mammals including the menstrual cycle in women (Sacks, 1993). Interestingly, association between caffeine intake and oestradiol and/or oestrone level has been reported in women (Ferrini and Barrett-Connor, 1996). Furthermore, bio flavonoids are known to possess intrinsic oestrogenic activity (Sacks, 1993) and some tea flavonoids are reported to bind to oestrogen receptors (Marsden and Messonnier, 2007). Since BTB contains both caffeine and flavonoids (Balentine *et al.*, 1997; Modder and Amarakoon, 2002) a possibility exists that regular heavy consumption of these may disrupt oestrous cycle and/or menstrual cycle. But, this has not been scientifically examined and reported sofar.

The aim of this study was to examine the effects of BTB on oestrous cycle with a view to extrapolate data obtained to the menstrual cycle. This was tested using Sri Lankan high grown Dust grade No: 1 tea and regularly cycling female rats. The Dust grade was selected since it is the most widely consumed by Sri Lankans tea drinkers.

MATERIALS AND METHODS

Animals

Laboratory bred healthy adult females Wistar rats (weighing 200-225 g) showing regularly cycling vaginal cycles 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.

Preparations 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 (low dose: 84 mg/ml, equivalent to 1.5 cups; mid dose: 168 mg/ml, equivalent to 3 cups and high dose: 501 mg/ml, equivalent to 9 cups) were made in 2 ml of water. The volume of 1 cup is considered as 170 ml.

Effects on oestrous cycle

Thirty six female rats were randomly assigned into 4 equal groups (N = 9/group). Daily vaginal smears were taken for 10 days from these rats (8.00 - 9.00 h) using 50 µl of isotonic saline (0.9 % NaCl) and the stage of the vaginal cycle was recorded microscopically (at x100): prooestrous, oestrous and dioestrous (Ratnasooriya and Dharmasiri, 1998). From day 11 to 42 these rats were daily orally administered with BTB in the following manner: group 1 with low dose, group 2 with mid dose, group 3 with high dose and group 4 with 2 ml of vehicle (water). Two hours after oral treatment, these rats were subjected to vaginal smearing and the stage of the oestrous cycle was recorded. During the treatment period these rats were observed daily for overt signs of toxicity (salivation, diarrhoea, teeth exposure, lachrymation, ptosis, squint, stupor, tremors, ataxia, rolling, jumping, yellowing of hair, loss of hair, postural abnormalities and behavioural changes), stress (fur erection or exopthalmia) and aversive behaviours (biting of paw, intense grooming behaviour, scratching behaviour, licking of tail or vocalization), morbidity and mortality (Ratnasooriya and Jayakody, 2006).

Statistical analysis

Data are given as means ± SEM. Statistical comparisons were made using the Mann-Whitney U-test and λ^2- test as appropriate. $P \leq 0.05$ was considered as indicating significance.

RESULTS

The results obtained with respect to oestrous cycle are summarized in Tables 1 and 2. As shown in Table 1, none of the doses of BTB significantly ($P > 0.05$) altered the percentage occurrence of different stages of the oestrous cycle: prooestrous, oestrous and dioestrous. The oral treatment of BTB also did not significantly ($P > 0.05$) alter the length of the oestrous cycle (Table 2). Further, none of the dose of BTB induced overt signs of toxicity, stress, aversive behaviours, morbidity or mortality.

DISCUSSION

This study examined the effect of black tea brew on oestrous cycle of rat with a view to extrapolate the data obtained to the menstrual cycle of women: oestrous cycle of rat has

Table 1. Effect of oral administration of black tea brew (BTB) of *Camellia sinensis* on the oestrous cycle of rats (Mean ± SEM)

Treatment	Pre treatment (10 days)			Treatment (31 days)		
	% occurrence of different oestrous stages					
	Prooestrous	Oestrous	Dioestrous	Prooestrous	Oestrous	Dioestrous
Control (2 ml water)	25.0 ± 1.39	20.0 ± 0.10	55.0 ± 1.39	26.0 ± 0.54	17.8 ± 0.56	56.1 ± 1.28
Low dose BTB (eq. 1.5 cups)	23.3 ± 0.86	25.0 ± 0.91	51.6 ± 1.25	27.1 ± 0.73	18.3 ± 0.68	54.4 ± 0.75
Mid dose BTB (eq. 3 cups)	23.3 ± 0.68	21.6 ± 0.68	55.0 ± 0.91	24.9 ± 0.67	17.8 ± 0.56	57.7 ± 0.73
High dose BTB (eq. 9 cups)	23.3 ± 0.86	26.6 ± 0.86	50.0 ± 1.17	24.9 ± 0.67	18.8 ± 0.47	56.1 ± 0.73

eq. = equivalent

many similarities with the menstrual cycle except for the bleeding that occurs at the beginning of the menstrual cycle (Talbert, 1978; Sacks, 1993). The results show, for the first time, that daily administration of black tea brew (even upto to 9 cups) for a period of one month dose not disrupt the oestrous cycle of rats both in terms of percentage occurrence of different stages of oestrous cycle, namely, prooestrous, oestrous and dioestrous and the duration of the oestrous cycle. This observation also suggests that ovulation and reproductive hormones which regulate the ovulatory cycle are unlikely to have affected by BTB administration. The one month treatment period employed in this study is an adequate interval as the duration of the oestrous cycle in rat is 4-5 days (Talbert, 1978) which was evident in this study as well. If the results are applicable to women then this finding may possibly indicates that even with heavy daily consumption of black tea brew for long periods the menstrual function is unlikely to be altered markedly. In contrast, heavy consumption of caffeine, which is rich in both coffee and tea is known to shorten the duration of the menstrual cycle (Fenster *et al.*, 1999) and menstruation (Fenster et al., 1999) in women. The undisturbed oestrous cycle evident in this study even with heavy administration of BTB, which contains appreciable amounts of caffeine and flavonoids is an important finding of clinical significance as both these compounds have the potential to interrupt oestrous cycle (Sacks, 1993; Fenster *et al.*, 1999). The exact reason for this un

Table 2. Effect of oral administration of black tea brew (BTB) of *Camellia sinensis* on the oestrous cycle of rats (Mean ± SEM)

Treatment	Length per cycle (days)	
	Pre treatment (10 Days)	Treatment (31 days)
Control (2ml water)	5.33 ± 0.33	5.44 ± 0.27
BTB		
Low dose (eq. 1.5 cups)	5.14 ± 0.14	5.81 ± 0.22
Mid dose (eq. 3 cups)	5.33 ± 0.21	5.65 ± 0.20
High dose (eq. 9 cups)	5.28 ± 0.18	5.18 ± 0.19

eq. = equivalent

interruption of the oestrous cycle is unknown at present but may probably be due to effects of other phytoconstituents present in BTB. Black tea brew of *C. sinensis* contains a variety of phytoconstituents (Balentine *et al.*, 1997; Modder and Amarakoon, 2002). Studies have shown that some chemical components present in black tea can modify the pharmacological effects of caffeine (Abeysinghe, 2003). Alternatively, caffeine may not have any effects on the length of the oestrous cycle and variability (Cooper *et al.*, 1996).

In conclusion, this study demonstrates for the first time that heavy dose of BTB, even if administered daily, dose not affect the oestrous cycle in rats.

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