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Effect of Sri Lankan black tea brew *(Camellia sinensis)* on hexabarbital induced sleep in mice

W.D. Ratnasooriya, T.S.P. Fernando and P.P. Madubashini
Department of Zoology, University of Colombo, Colombo 03, Sri Lanka.

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Abstract

Present study examined the effect of warm black tea brew/ infusion (BTB) of *Camellia sinensis* (L.) O. Kuntze (family: Theaceae) on sleep. The above was tested in hexabarbital induced sleep model in mice using three doses (equivalent to 1.5. 3. and 9 cups. 1 cup 170 ml) of Sri Lankan high grown Dust grade No: 1 black tea or a single dose (equivalent to 9 cups) of green tea brew (GTB) of Japanese and Chinese varieties. The results showed that black tea brew (BTB) significantly ($P < 0.05$) and dose-dependently prolonged the onset and shortened the duration of sleep. A similar effect was elicited with the high dose of GTBs but with a higher efficacy. Decaffeination of BTB suppressed the effects on the onset of sleep induced by the normal BTB. It is concluded that Sri Lankan black tea disrupts sleep in mice possibly via dopaminergic, serotoninergic and nerve stimulant mechanisms.

Key words - *Camellia sinensis;* sleep; theanine; caffeine; tea; dust grade

1. Introduction

Tea which is prepared from the topmost immature leaves and buds of the perennial evergreen shrub *Camellia sinensis* (L.) O. Kuntz (Family: Theaceae) is the most widely consumed drink in the world, besides water (Modder and Amarakoon. 2002). Infact, it is one of the healthiest beverages today. 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 semi fermented).

Of these types, black tea accounts for about 78% of world tea production and about 80% of global tea consumption (Modder and Amarakoon, 2002). However, the amount of research done on black tea, especially, on its bioactivity is limited compared to green tea which is consumed only by about 16% of the world population (Ho, et al., 2005). Regrettably, very little research has been done on bioactivity of Sri Lankan black tea although Sri Lanka is the second most major tea producing country in the world (Anonymous, 2006). Hence, it is reasonable to focus more efforts on health effects of Sri Lankan tea. Further, it is known that several factors such as country of origin, geological background of soil, the

elevation of the tea plantation, collecting season, technological processes during tea production, brewing conditions influence the final chemical composition of tea brew (Gramza, et al., 2006) and hence its pharmacological effects.

The study reported herein examines the of effect Sri Lankan black tea on sleep. This was investigated in mice using hexabarbital induced sleep model and high grown Sri Lanka Dust grade No I black tea. The dust grade was selected as it is the most widely consumed type of tea by Sri Lankan tea drinkers probably because of its comparatively low price, characteristic flavour and easy availability.

2. Materials and methods

Experimental Animals

Healthy adult ICR strain male mice (weight: 35-40 g) purchased from the Medical Research Institute, Boralla, Sri Lanka were used. They were housed under standardized animal house conditions with free access to pelleted food and water *ad libitum.* All animal 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, for animal experimentations. For ethical reasons minimum number of animals were used.

Source of tea

Two or three topmost immature leaves and buds of C. *sinensis* plucked from the plantation of St. Coombs tea estate of the Tea Research Institute. Sri Lanka (1382 m above sea level: high grown) in August 2005 was used to process Dust grade No.l black tea by orthodox-rotorvane technique at the estate factory. Green tea was also made in the same factory by subjecting the shoots to heat either by steaming (Chinese type) or dropping on to a heated pan (Japanese type) and then bypassing the typical fermentation and drying processes. Decaffeinated black tea was made as described by Pavia et al (1976). Briefly, to 100 g Dust grade No: 1 tea 1 L of boiling distilled water was added and allowed to stand for 5 min. The solution was then filtered through muslin cloth to remove solid particles and allowed to cool. Caffeine was removed by chloroform extraction (2 x 50 ml) using a seperatory funnel. The aqueous layer was separated and freeze dried (Pavia, et al., 1976).

The tea sample selected was pure, unblend and typical to the grade as confirmed by sieve analysis, organoleptic profile, and physical and chemical analysis.

Preparation of tea brew

Black tea brew (BTB), green tea brew (GTB) and decaffeinated tea brew (DTB) were made by adding 2 g of respective tea samples to 100 ml boiling water and brewed for 5 minutes (Anonymous. 1980) [yield (w/w) for BTB: 43.7%: GTB (Chinese type): 49.5%: GTB (Japanese type): 46.6%; decaffeinated BTB: 43.7%].

For oral treatment of mice, 3 doses of BTB (84mg/ml, equivalent to 1.5cups: 167mg/ml, equivalent to 3 cups; and 501 mg/ml, equivalent to 9 cups) a single dose each of GTBs Chinese type (600 mg/ml, equivalent to 9 cups) and Japanese type (634 mg/ml. equivalent to 9 cups) and a single dose of decaffeinated BTB (501 mg/ml. equivalent to 9 cups) were made in 0.5 ml of water. The volume of one cup is considered to be 1 70 ml.

Evaluation of the hexabarbital induced sleeping time

Sixty three mice were randomly assigned in to seven equal groups (N= 9/group). These mice were orally treated with warm tea brew (37 °C) in the following manner: group 1, 0.5 ml of water; group 2, with low dose of BTB: group 3, with mid dose of BTB: group 4, with high dose of BTB: group 5, with high dose of Chinese type of GTB; group 6. with high dose of Japanese type of GTB; group 7, with high dose of decaffeinated BTB. One hour later, these mice were intraperitoneally injected with 75 mg/kg dose of hexabarbital (Dhawan and Srimal, 1984). The time for onset of sleep and the duration of sleep were recorded. The criterion used for the onset of sleep was the loss of righting reflex and for the emergence of awakeness was the appearance of righting reflex.

Statistical analysis

The results are expressed as means ± SEM. Statistical comparison were made using Mann-Whitney U test. Dose dependencies are determined by using Pearson's correlation test. Significance was set at $p \leq 0.05$.

3. Results

The results are summarized in Table 1. As shown, all the doses of BTB tested significantly ($P < 0.05$) and markedly prolonged the time of onset of sleep (low dose by 66%; mid dose by 83%; and high dose by 374%) and shorten the duration of sleep (low dose by 68%; mid dose by 66%; high dose by 77%). These effects were dose-related (onset, $r^2 = 0.87$ $P < 0.05$ and duration, $r^2 = 0.89$; $P < 0.05$). The high dose of GTB also profoundly and markedly increased the time of onset of sleep (Chinese by 284% and Japanese by 158%) and suppressed its duration (Chinese by76% and Japanese by 57%). In contrast, decaffeination of black tea significantly, ($P < 0.05$) reduced the time of onset of sleep and its duration.

4. Discussion

The results of this study clearly show that BTB made from Sri l.ankan high grown Dust grade No: 1 black tea prolonged the onset and shortened the duration of hexabarbitone-induced sleep in mice. This effect was dose-dependent possibly indicating receptor mediation. A similar pattern of response but with a higher efficacy was evident with both Chinese and Japanese types of green teas.

This tea-induced dual effect on sleep suggests that consumption of tea may actually disrupts the quality of sleep. This is incontrast to general belief that drinking tea prior to sleep facilitates the onset and prolongs the duration of sleep. However, it could be argued that the study reported here was done using a drug induced experimental paradigm and may not be valid for spontaneous sleep. But, working with conventional spontaneous sleep models with rodents is practically difficult and drug-induced rodent sleep models are used widely in evaluating drug effects on sleep and insomnia (Borbely. 1983) and therefore the results obtained are valid and meaningful.

Mice show a sleep-wake cycle like humans (Borbely, 1983). Most scientists agree that this cycle is regulated by serotonergic, catecholaminergic and cholinergic neurones innervating certain nuclei in the hypothalamus and reticular activating system of brain (Borbely, 1983; Saladin, 2004). Further, dopamine is known to cause wakefulness and arousal (Borbely, 1983; Saladin, 2004). Tea is shown to raise the dopamine level in brain via theanine (Yokogoshi, 1998), [an amino acid unique to tea (Modder and Amarakoon, 2002)] and this could account as one possible mechanism for suppression of sleep in this study. Theanine in tea also elevates serotonin level in brain (Yokogoshi, 1998). Reduction in brain serotonin level cause insomnia and it could be reversed by serotonin (Borbely, 1983). Tea induced escalation of serotonin levels should promote sleep but such an effect was not evident in this study. The inability of tea to increase serotonin level in brain sufficiently to trigger sleep could be the reason for the failure. On the other hand, it is noteworthy that one of the commonest side effects of selective serotonin reuptake inhibitors (which increases brain serotonin level) used in depression is insomnia (Anonymous, 2000). A strong possibility exists that a similar mode of action is operative with tea. there by inducing a delay in onset and shortening the duration of sleep.

Both black and green tea contains caffeine (Modder and Amarakoon, 2002) which is a well recognized mild nerve stimulant, which increase alertness (Modder and Amarakoon, 2002). It is quite possible that this stimulatory activity could account for the tea-induced deficit in sleep observed in this study. This notion is supported by the fact that decaffeination of black tea triggered a shortening of time to onset of sleep. However, unexpectedly, decaffeination did not prolonged the duration of sleep.

In addition, especially, at high dose, tea can bring about non specific pharmacological effects to interfere sleep because tea is highly rich in phytochemicals (Modder and Amarakoon, 2002) which could act synergisticaly and dysergistically. Further experiments are however, needed to clarify these potential mechanisms.

In conclusion, this study show, for the first time that Sri Lankan black tea disrupts the onset and duration of sleep. This is an important finding of clinical relevance.

5. Acknowledgements

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Table 1: Effect of black tea, decaffeinated black tea and green tea on hexabarbital- induced sleeping time in mice (mean ± SEM)

Treatment	Parameters	
	Onset of sleep (min)	**Duration of sleep (min)**
Control (water)	21.75 ± 0.60	73.75 ± 2.56
Low dose of BTB (eq. 1.5 cups)	36.08 ± 0.81*	24.00 ± 0.38*
Mid dose of BTB (eq. 3.0 cups)	39.91 ± 2.24*	24.91 ± 0.99
High dose of BTB (eq. 9.0 cups)	103.25 ± 4.99*	15.91 ± 0.50*
High dose of Chinese type GTB (eq. 9.0 cups)	83.50 ± 3.19*	17.75 ± 0.93*
High dose of Japanese type GTB (eq. 9.0 cups)	56.66 ± 1.75*	31.75 ± 1.59
High dose of Decaffeinated BTB (eq. 9.0 cups)	12.16 ± 0.27*	18.25 ± 0.66*

* $P < 0.05$ compared to control (Mann-Whitney U- test)
eq. = equivalent: BTB = Black tea brew: GTB = Green tea brew