



SRI LANKAN ORANGE PEKOE GRADE BLACK TEA IMPAIRS ANXIETY

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Abstract: There is an unmet need for the development of new herbal pharmaceuticals as anxiolytics. In this context, the present study, investigated the anxiolytic potential of warm black tea brew/ infusion (BTB) of Sri Lankan low grown orthodox Orange Pekoe (O.P.) grade tea made from *Camellia sinensis* (L) O. Kuntz (Theaceae). This was tested in rats using three models of anxiety (hole-board, neophobic suppression of eating and drinking tests). Different doses [223 mg/kg (equivalent to 1.5 cups), 446 mg/kg (equivalent to 3 cups) and 1339 mg/kg (equivalent to 9 cups)] or water was orally administered to rats (N = 6-10/group) and anxiolytic effects were determined after 1h. The results showed that BTB possesses dose-dependent anxiolytic action with quick onset (1h). This anxiolytic action of BTB was not accompanied with undesirable side effects: muscle relaxation, motor in co-ordination or sedation. In addition, BTB produced no overt signs of toxicity. BTB induced significant increase in the number of entries, time spent in open arms, % open arm entries and % time on open arms and reduced the time spent in closed arms when tested in elevated plus maze model. This suggest that main mode of anxiolytic action of BTB is GABAergic. It is also suggested that, serotonergic and dopaminergic mechanisms also play a part in mediating anxiolytic action. It is concluded that, O.P. grade Sri Lankan black tea has oral anxiolytic action as claimed in Sri Lankan traditional medicine and consumption of at least 3 cups of BTB may be useful to impair 'day to day' anxiety.

Keywords: Anxiety, Anxiolytic, Tea, Orange Pekoe Grade Tea, Sri Lankan Tea, GABAergic, Serotonergic, Dopaminergic

INTRODUCTION

Anxiety is an emotional state, unpleasant in nature and is associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat⁽¹⁾. It is a wide spread phenomenon and its prevalence is mounting⁽²⁾. Currently, anxiety affects one eighth of the worldwide population⁽²⁾. The prevalence of anxiety is higher in women compared to men⁽²⁾ and its occurrence is greater in young people⁽²⁾. It is estimated that, up to 40% of individuals would experience an anxiety disorder in their life time and up to 5% would develop chronic anxiety disorders⁽²⁾. In the allopathic medicine, several drugs are currently available for the treatment of anxiety^(3,4) and these act mainly via GABAergic, serotonergic and dopaminergic pathways^(3,4). Although, these drugs are efficacious, are accompanied with unpleasant side effects. These include sedation, insomnia, dizziness, nausea, drowsiness, tremor, nervousness, headache, muscle relaxation, salivation changes, gastro-intestinal disturbances, sexual dysfunction, addiction, tolerance and withdrawal⁽³⁾. In addition, these drugs are expensive and not easily available, especially in developing countries. Furthermore, these drugs may impair performance of skilled tasks such as driving⁽³⁾. Hence, there is a greater deal of popular and medicinal interest in the use of dietary supplements and nutraceuticals in order to manage normal "every day"

stress and anxiety⁽⁵⁾. Interestingly, there is a wide range of all-natural non-prescription formulations currently available in the market to ease "every day" anxiety and stress⁽⁵⁾. These are claimed to contain several nutraceutical compounds and herbal extracts and are available in the form of pills (e.g. RelarinTM, SeditolTM) and powders for mixing into drinks (e.g. chamomile, kava kava)⁽⁵⁾.

In this regard, we recently investigated the anxiolytic potential of hot water brew/infusion (BTB) of orthodox high grown Sri Lankan black tea manufactured from dried tender terminal leaves and bud of the plant *Camellia sinensis* (L) O. Kuntz (Family: Theaceae)⁽⁶⁾: since, some reputed Sri Lankan traditional practitioners and herbalists claim that consumption of 3-5 cups of warm black tea help to relieve anxiety. Black tea is the most consumed beverage in the world second to water⁽⁷⁾ and accounts for about 78% of the world tea production and about 80% of the global tea consumption⁽⁷⁾. Its per capita consumption is 2.52 – 3.1 kg/person/annum⁽⁸⁾. The grade of tea used in this study⁽⁶⁾ was high grown (above 1200m, average mean sea level) Dust No: 1 and the testing was done in rats. There are two main categories of orthodox black tea: broken grade and whole grade. Dust No: 1 belongs to broken grade. However, the anxiolytic potential of whole leaf grade



category is not investigated so far. Further, it is known that bioactivity of black tea depends on particle size and agroclimatic elevation amongst other things^(9, 10). Hence, this study was launched to evaluate the anxiolytic potential of Sri Lankan low grown (below 600m, average mean sea level) whole leaf grade black tea using Orange Pekoe (O.P) (1400 μm – 2000 μm) tea.

MATERIALS AND METHODS

Experimental animals:

Healthy adult male Wistar rats (200-250g) purchased from the Medical Research Institute, Colombo, Sri Lanka and bred in the animal house of the Department of Zoology, University of Colombo were used. They were kept under standardized animal house conditions (temperature: 28-31 °C, photoperiod: approximately 12 hours natural light per day, relative humidity: 50-55%) with free access to water and pelleted food (Master Feed Ltd., Colombo, Sri Lanka) at the animal house of the Department of Zoology, University of Colombo. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care guidelines⁽¹¹⁾ and guidelines and rules of the Faculty of Science, University of Colombo for animal experimentations.

Source of tea:

Topmost immature leaves and buds of *C. sinensis* plucked from the plantation of St. Jochims tea estate of the Tea Research Institute, Hedallana, Ratnapura, Sri Lanka (29 m above mean sea level: low grown) during November – December 2011 were used to process O.P. grade black tea by orthodox-rotovane technique at the estate factory. The composition of true to size particles defined for the O.P. grade black tea was determined using sieve shaker (Retsch AS 200, Retsch GmbH, Haan, Germany) with standard set of sieves (shaking time: 10 minutes and shaking speed: 50 vibrations/minute). Typical characters belonging to elevations were assessed organoleptically by professional tea tasters of the Tea testing unit, Sri Lanka Tea Board. 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 the ISO standards⁽¹²⁾ adding 2g of O.P. grade black tea to 100 ml of boiling water and brewed for 5 min. This contained 36.1% (w/w) tea solids in water. Based on this data, 1339 mg/kg (equivalent to 9 cups/ high dose, 1 cup = 150 ml) of BTB in 3 ml of water was prepared by adding 10 g of O.P. grade black tea to 30 ml boiling water and brewed for 5 min. Then 446 mg/kg (equivalent to 3 cups/ mid dose) and 223 mg/kg (equivalent to 1.5 cups/ low dose) doses of BTB were prepared by diluting appropriately

with boiling water. Slightly warm BTB (40 ± 3 °C) was used in oral administration.

Evaluation of anxiolytic activity using rat hole-board technique:

Thirty eight rats were randomly divided into 4 groups and treated orally in the following manner; Group 1: 3 ml of water (control / N = 9), Group 2: 223 mg/kg dose of BTB (N = 10), Group 3: 446 mg/kg dose of BTB (N = 9), Group 4: 1339 mg/kg dose of BTB (N = 10). Then rats were kept in their original cages. One hour post treatment, each rat was individually placed in the center of a standard rat hole-board apparatus and observed for 7.5 min (in between 09. 00 – 11.00 h). During this period, the number of rears, locomotory activity in terms of number of crossings, number of head dips, total dipping time and number of fecal boluses expelled were monitored. The time spent for a head dip was then computed⁽¹³⁾.

Evaluation of food intake in a novel environment:

Twenty four rats were starved for 16 hours and randomly divide in to four groups (N = 6/group). Then they were orally treated with water (control) or BTB of O.P. grade black tea in the following manner: Group 1: 3 ml of water, Group 2: 223 mg/kg dose of BTB, Group 3: 446 mg/kg dose of BTB, Group 4: 1339 mg/kg dose of BTB. After that, they were kept in their respective original cages. 1h post treatment they were individually placed in a wooden box (35 × 60 × 60 cm) containing weighed amount of novel food (dry fish). After 30 min exposure to the novel environment, the rats were removed and the amount of food consumed was measured⁽¹⁴⁾. In this test, increased intake of novel food in an unfamiliar environment is considered as a measure of anxiolytic activity. This experiment was conducted between 09.00-11.00h.

Evaluation of sweetened condensed milk intake in a novel environment:

Twenty four rats were starved for 16 hours and randomly divide in to four groups (N = 6/group). Then they were orally treated with water (control) or BTB of O.P. grade black tea in the following manner: Group 1: 3 ml of water, Group 2: 223 mg/kg dose of BTB, Group 3: 446 mg/kg dose of BTB, Group 4: 1339 mg/kg dose of BTB. After that, they were kept in their respective original cages. 1h post treatment they were individually placed in a wooden box (35 × 60 × 60 cm) containing a bottle with 50 ml of sweetened condensed milk (sweetened condensed milk: water – 1:1). After 2 h exposure to the novel environment, the rats were removed and the amount of milk consumed was measured⁽¹⁵⁾. This experiment was conducted between 09.00 – 11.00h.

Evaluation of the GABAergic activity using the elevated plus maze model:

Sixteen rats were randomly divided in to two equal groups (N = 8/group) and one group was treated with 3 ml water (control) and the other with 446 mg/kg dose of BTB of O.P. grade black tea. Then they were kept in their original cages. 1h post treatment rats were individually placed in the neutral zone facing to an open arm of a wooden elevated plus maze model [consist of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) extending from a central area (neutral zone) of 10 × 10 cm; elevated to a height of 50 cm] and observed for 5 min (in between 09. 00 – 11.00h). During this period, number of entries to open arms, time spent in open arms, number of entries to closed arms, time spent in closed arms, number of entries to the neutral zone and time spent in the neutral zone were monitored. Then total number of entries (number of entries to open arms + number of entries to closed arms), % open arm entries (number of open arm entries / total number of entries × 100) and % time spent in open arms (time spent in open arms / total time spent in open arms and closed arms × 100) were computed⁽¹⁶⁾.

Evaluation of muscle strength and neuro-motor coordination:

Eighteen rats were randomly divided in to two groups and treated orally in the following manner; Group 1: 3 ml of water (control / N = 9), Group 2: 446 mg/kg dose of BTB (N = 9). Then rats were kept in their original cages. One hour post treatment, each of these rats were subjected to bar holding test to evaluate the muscle strength⁽¹⁷⁾, Bridge test⁽¹⁷⁾ and righting reflex test⁽¹⁸⁾ to evaluate the neuro-motor coordination and their respective latencies were recorded.

Evaluation of acute adverse effects:

All rats used to determine the anxiolytic activity in the rat hole-board test were closely observed for 2-3 h for any overt signs of toxicity (diarrhoea, jumping, restlessness, salivation, rhinorrhea, lachrymation, chewing jaw movements, exposure of teeth, ptosis, squint, writhing, convulsions, tremors, ataxia, rapid

rotational movements of head, neck and/or entire body around the spinal axis, pallor of lips, flat body posture, walking backwards, impairments of auto grooming or excessive grooming, cleaning of face, breathing movements of chest, lethargy and sleepiness), stress (fur erection and exophthalmia), or aversive behaviors (biting and scratching, licking of tail, paw and penis or vocalization).

Statistical analysis:

The results are expressed as means ± SEM (Standard Deviation of Mean). Statistical comparisons were made using Mann-Whitney U test⁽¹⁹⁾ using Minitab 14.0 statistical package. Significant level was set at P < 0.05.

RESULTS

Sieve analysis and organoleptic analysis:

Sieve analysis revealed that 83.5% of tea particles were true size (2000 – 1400 μm) for O.P. grade black tea. This indicates the tea sample used in the study was typical to O.P. grade black tea. Organoleptic testing by professional tea testers showed that the sample can be considered as well made high quality low grown O.P. grade Sri Lankan black tea.

Anxiolytic activity in rat hole-board test:

As shown in the Table.1, all three doses of BTB of O.P. grade black tea significantly (P < 0.05) increased the number of crossings (low dose by 43%, mid dose by 54% and high dose by 50%) and total dipping time (low dose by 123%, mid dose by 65% and high dose by 114%). This effect on locomotor activity was dose dependent ($r^2 = 1.0$, P < 0.05). On the other hand, only mid dose (by 58%) and high dose (by 58%) significantly (P < 0.05) increased the number of rears, whilst the low dose non-significantly (P > 0.05) increased (by 26%) the number of rears. In complete contrast, none of the doses of BTB significantly (P > 0.05) altered the time/dip and number of fecal boluses.

Table.1: Effect of Orange Pekoe grade black tea on parameters of rat hole-board (Mean ± SEM)

Dose	Number of Crossings	Number of Rears	Number of Head Dips	time/dip (s)	Number of fecal bolus
Control (N = 9)	20.2 ± 2.3	21.6 ± 2.4	5.8 ± 1.2	1.11 ± 0.16	2.4 ± 0.8
1.5cups (223 mg/kg) (N = 10)	29.0 ± 2.2 *	27.4 ± 2.4	8.6 ± 1.2	1.44 ± 0.16	0.9 ± 0.4
3 cups (446 mg/kg) (N = 9)	31.2 ± 3.3 *	34.2 ± 1.7 **	7.5 ± 0.8	1.24 ± 0.09	1.6 ± 0.8
9 cups (1339 mg/kg) (N = 10)	30.4 ± 2.4*	34.2 ± 1.5 **	7.7 ± 1.3	1.44 ± 0.19	2.4 ± 0.7

*- P < 0.05; compared to control (Mann-Whitney U test)

Food intake in a novel environment:

The results are summarized and depicted in Table.2. As shown, all three doses of BTB of O.P. grade black tea increased the food intake (low dose by 25%, mid dose by 91% and high dose by 16%). However, the effect was significant ($P < 0.05$) only with the mid dose. Nevertheless, the effect of food intake in a novel environment exhibited a curvilinearly dose dependent ($r^2 = 1$) trend.

Table.2: Effect of Orange Pekoe grade black tea on food intake in a novel environment (Mean \pm SEM)

Dose	Food intake (g)
Control (N = 6)	0.410 \pm 0.046
1.5 cups (223 mg/kg; N = 6)	0.516 \pm 0.017
3 cups (446 mg/kg; N = 6)	0.784 \pm 0.069 *
9 cups (1339 mg/kg; N = 6)	0.474 \pm 0.026

*- $P < 0.05$; compared to control (Mann-Whitney U test)

Sweetened condensed milk intake in a novel environment:

The results are summarized in the Table.3. As shown, all three doses of BTB of O.P. grade black tea increased the milk intake (low dose by 35%, mid dose by 66% and high dose by 25%). Like with food intake, significant ($P < 0.05$) increase in the milk intake was evident only with the mid dose. Nevertheless, a curvilinearly dose dependent ($r^2 = 1$) trend was seen in milk intake.

Table.3: Effect of Orange Pekoe grade black tea on sweetened condensed milk intake in a novel environment (Mean \pm SEM)

Dose	Milk intake (ml)
Control (N = 6)	15.3 \pm 2.76
1.5 cups (223 mg/kg; N = 6)	20.8 \pm 0.99
3 cups (446 mg/kg; N = 6)	25.5 \pm 1.4 *
9 cups (1339 mg/kg; N = 6)	19.2 \pm 1.89

*- $P < 0.05$; compared to control (Mann-Whitney U test)

GABAergic mechanism in elevated plus maze model:

The results are summarized in the Table.4. As shown, the mid dose of BTB of O.P. grade black tea markedly and significantly ($P < 0.05$) increased the number of entries to open arms (by 111%), the time spent in open arms (by 129%), % open arm entries (by 51%), % time spent in the open arms (by 94%), number of entries to the neutral zone (by 63%), and significantly ($P < 0.05$) decreased the time spent in closed arms (by 27%). In contrast, time spent in the neutral zone and number of entries to closed arms was not significantly ($P > 0.05$) altered.

Muscle strength and neuro-motor coordination:

Mid dose of BTB of O.P. grade black tea did not significantly ($P > 0.05$) alter the latencies in bar test (control vs treatment: 33.89 \pm 6.55 vs 36.33 \pm 7.39), Bridge test (control vs treatment: 20.33 \pm 3.68 vs 18.61

\pm 5.65) and righting reflex test (control vs treatment: 1 \pm 0.0 vs 1 \pm 0.0).

Table.4: Effect of Orange Pekoe grade black tea on activity in elevated plus maze model (Mean \pm SEM)

Parameter	Control (N = 8)	3 cups (446 mg/kg) (N = 8)
Time spent in open arms (s)	27.1 \pm 6.4	62.2 \pm 12.6 *
No. of entries to open arms	2.3 \pm 0.7	5.0 \pm 0.5 *
Time spent in closed arms (s)	237.0 \pm 10.2	173.5 \pm 17.2 *
No. of entries to closed arms	5.5 \pm 0.7	7.0 \pm 0.4
Total No. of entries	7.8 \pm 1.2	12.0 \pm 0.8 *
% open arm entries	27.3 \pm 4.5	41.2 \pm 2.4 *
% time spent in open arms	14.7 \pm 4.1	28.6 \pm 5.3 *
Time spent in neutral zone (s)	35.8 \pm 4.2	46.0 \pm 4.0
No. of entries to the neutral zone	6.7 \pm 1.3	11.0 \pm 0.8 *

*- $P < 0.05$; compared to control (Mann-Whitney U test)

Evaluation of acute adverse effects:

Acute oral treatment of none of the doses of BTB induced overt signs of toxicity, stress or aversive behaviors. Further, none of the treated rats showed signs of morbidity and died during the observation period.

DISCUSSION

This study examined the anxiolytic potential of warm BTB made from Sri Lankan low grown orthodox O.P. grade black tea in rats. Rats are widely used in evaluating anxiolytic and anxiogenic activities of potential drugs^(6,16,18). The BTB was made using a typical sample (as determined by organoleptic and sieve analysis) of garden fresh and unblended O.P. grade tea sample. This is an important feature of this study as it is known that bioactivity of black tea varies with country of origin, grade of tea, particle size and agroclimatic elevation^(9,10). Most of the previous studies on bioactivities of black tea have used blended tea of multiorigin or unknown origin purchased from supermarkets⁽²⁰⁾. For the preparation of BTB, five minutes brewing time was used as indicated in ISO standard⁽¹²⁾: as extraction of water soluble flavanoids (flavanols, theaflavins and thearubigins) is almost complete within four minutes⁽²¹⁾. Warm (40 \pm 3°C) BTB was used for oral administration as cooling results in precipitation of important phytochemicals such as flavanoids and caffeine⁽²¹⁾. For testing the anxiolytic potential of BTB, three widely used, reliable, sensitive, well established and validated models of anxiety were used as in our previous study⁽⁶⁾: rat hole-board⁽¹³⁾, neophobic suppression of food intake⁽¹³⁾ and condensed milk intake⁽¹⁵⁾. All these three tests are based on measurements of behavioral inhibitions (considered to reflect anxiety) in response to novelty.

The results show, for the first time, that BTB of Sri Lankan low grown orthodox O.P. grade black tea possesses marked anxiolytic activity (in terms of

increased number of rears, crossings in the hole-board and increase in food and milk intake in neophobic tests). This BTB induced anxiolytic activity had a quick onset (within 1h) indicating that the action is mediated via a phytoconstituent/s already present in the BTB and not through their metabolite/s. This anxiolytic action of BTB of O.P. grade tea is almost comparable to what is already reported for Sri Lankan high grown orthodox Dust grade No: 1 tea⁽⁶⁾. As mentioned earlier in the introduction section, orthodox black tea of all grades falls into one of two categories, namely, broken grade and whole grade. Taken together, the results of our previous study⁽⁶⁾ and this study suggest that consumption of few cups of orthodox black tea, irrespective of the grade, is a useful alternative in the management of anxiety of everyday life. Also, these two studies scientifically substantiate the claims made by some Sri Lankan traditional practitioners that black tea has anxiolytic potentials. It is of interest to note that a typical tea drinker consumes about 3 cups daily and the maximum recommended amount is 10 cups⁽⁷⁾.

The BTB induced anxiolytic activity was dose-dependent. This indicates that the effect is genuine, intrinsic, causal, specific and possibly receptor mediated. It is well recognized that GABA pathways play a critical role in the etiology of and treatment of anxiety^(4,22): low levels of GABA in the brain are associated with anxiety^(5,23) and drugs that agonize the GABA receptors either directly (e.g. Gabapentin) or indirectly via benzodiazepine receptors (e.g. Loxatonil) are used in the treatment of anxiety^(3,5). In this study, BTB of O.P. grade tea, compared to control, significantly increased the number of entries, the time spent in open arms, % open arm entries and % time spent on open arms and reduced the time spent on closed arms, when tested in elevated plus maze model. This indicates GABAergic mode of action of O.P. grade tea.

Nineteen amino acids are reported both in green and black teas⁽²⁴⁾. Of these L-theanine (γ -glutamylmethylamide) is unique to tea⁽²⁴⁾. Black tea provides 25-60mg of L-theanine per 200ml⁽²⁵⁾. L-theanine passes the blood-brain barrier and has been shown to potentiate the release of GABA on GABAergic synapses and increases GABA level in rat brains^(5,26). This mode of action is likely to act as the main mechanism of the anxiolytic action in this study as has been proposed for green tea⁽²⁷⁾ and Sri Lankan high grown orthodox Dust grade No: 1 black tea⁽⁶⁾. Black tea is a rich source of flavanoids such as flavanols, theaflavins and thearubigins⁽⁷⁾. Flavanoids are shown to bind to GABA_A receptors⁽⁵⁾ and this mechanism can also play a role in modifying the GABA signaling pathway to induce an anxiolytic action in this study. Extracts from GABAergic anxiolytic herbs and plants have been also shown to modify the GABA signaling

pathway by potentiating the GABA_A receptor expression⁽⁵⁾. However, it is unknown whether such a mode of action is operative in this study, but it is worth examining. Black tea contains appreciable amount of caffeine^(7,24) and caffeine shows weak to moderate affinities to both benzodiazepine receptors (which are linked to GABA receptors) and GABA_A receptors⁽²²⁾. Thus, a possibility exists that the BTB of O.P. grade tea could also mediate its anxiolytic action via this mechanism. However, such a mode of action is unlikely to be operative in this study as decaffeination of Dust grade black tea has not suppressed its anxiolytic action⁽⁶⁾.

It is now well recognized that monoamine neurotransmitter serotonin plays a vital role in regulation of anxiety^(4,5). Further, selective serotonin uptake inhibitors (SSRIs)^(3,4,5), serotonin receptor agonists^(3,4,5) and drugs which increase serotonin synthesis^(5,28) are used in the treatment of anxiety. L-theanine, which is known to cross the blood-brain barrier, has been shown to increase serotonin production in brain⁽²⁹⁾. Thus, L-theanine induced serotonin increase is also likely to play a pivotal role in producing anxiolytic action in this study. Currently, there is no evidence available to suggest that tea has selective serotonin inhibiting activity and/or serotonin agonist's action. However, these possibilities are worth examining.

Dopamine, yet another neurotransmitter in brain, is also claimed to play a role in anxiety^(3,4) and some drugs used in the treatment of anxiety mediate their action via dopaminergic pathways^(3,4). Interestingly, L-theanine in tea has shown to increase brain dopamine level and obviously BTB induced elevated dopamine level can also contribute to the anxiolytic effect observed in this study.

The anxiolytic action of BTB was not accompanied with muscle relaxation (as evident by bar test), motor incoordination (as judged by righting reflex and bridge tests), and sedation (as judged from hole-board test) which are common side effects of anxiolytic drugs⁽³⁾. Further, BTB did not induce overt signs of acute toxicity. Undoubtedly, lack of undesirable side effects is a plus point for O.P. grade black tea as an anxiolytic agent.

CONCLUSION

The present study demonstrates, for the first time, that BTB made from Sri Lankan low grown orthodox O.P. grade black tea has fast acting oral anxiolytic activity with no unpleasant side effects. Anxiolytic activity is possibly mediated via GABAergic, serotonergic and dopaminergic mechanisms. As claimed in Sri Lankan traditional medicine, regular consumption of at least 3 cups of black tea may be a

useful strategy to alleviate day to day anxiety resulting from present day stressful lifestyles.

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