ANTINOCICEPTIVE ACTIVITY OF SRI LANKAN BLACK TEA BREW (CAMELLIA SINENSIS L.) IN RATS
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ABSTRACT
The aim of this study was to investigate the antinociceptive potential of warm black tea brew of Camellia sinensis (L.) O. Kuntze (Theaceae). This was tested in rats using high grown Sri Lankan Dust grade No: 1 black tea and four models of noceiception (tail flick, hot plate, writhing and formalin tests). Four doses of black tea brew (BTB) 84, 167, 501, 1336 mg/ml was made and orally administed to rats (N = 9/group) for testing purposes. The results showed that strong BTB possesses marked and significant (P < 0.05) antinociceptive activity having a rapid onset and a short duration of action. Further, the BTB also had profound and significant (P < 0.05) antihyperalgesic activity (in terms of carrageenan induced thermal hyperalgesic test and formalin test), in vitro antioxidant activity (DPPH method) and antihistamine activity (in terms of wheal test). The BTB-induced antinociception was blocked by nalaxone but not by atropine or metachlopramide indicating opioid receptor mediation. Further, even with subchronic administration of BTB, tolerance was not developed. It is concluded that BTB of C. sinensis has mild to moderate oral antinociceptive activity with swift onset and short duration of action which is mediated via opioid, antioxidant and antihistamine mechanisms.

KEY WORDS: Antinociceptive; Black tea; Camellia sinensis; Dust grade tea; Sri Lankan tea

INTRODUCTION
Camellia sinensis (L.) O.Kuntze (Theaceae) popularly known, as the tea plant is an evergreen shrub or tree with leaves 5-9 x 2-2 cm, obtuse or short rounded points. It is native to Southeast Asia and is extensively cultivated in countries like India, Java, China, Japan, Bangladesh, Indonesia, Kenya, and Turkey for manufacture of tea. Tea is produced from freshly harvested tender shoots, comprising two or three of the topmost immature leaves and the bud of Camellia sinensis plant. Depending on the manufacturing technique there are three types of teas: black tea (fully aerated or fermented), green (unaerated or unfermented) and oolong (partially aerated or semi fermented). Phytochemically black tea contains flavonoids (catechins, theaflavins, thearubigins, flavonols), caffeine, amino acids including theanine, peptides, sugars, potassium, volatiles and vitamins.

Several scientific studies have indicated that tea drinking, particularly, the green teas, provide many health benefits: promotion of cardiovascular health; antioxidative; antiaging; anticarcinogenic; anti-inflammatory; antidiabetic; antibacterial; central nervous system stimulation; promotion of oral health, stress relieving and anxiolytic; diuretic. On the other hand, there are several traditional health claims for tea such as improving of blood flow, elimination of alcohol and toxins, clearance of urine and improvement of its flow, relieving of joint pains, improvement of resistance to diseases. According to Sri Lankan folkloric and traditional medicine strong black tea has pain relieving properties and often recommended for day-to-day body aches and pains, and joint pains. In addition, it is claimed to have diuretic action and this has been scientifically proven. However, pain killing property of tea has not been validated by scientifically conducted experimentations.

The aim of this study was to examine the antinociceptive potential of black tea. This was investigated in rats using high grown Sri Lankan Dust grade No: 1 black tea. The Dust grade was selected, as it is most widely consumed by Sri Lankan tea drinkers probably because of its comparatively low price, easy availability and flavour.

MATERIALS AND METHODS
Experimental animals
Healthy adults Wistar male rats (weight: 200-250 g) and female rats (weight: 175-200 g), and male mice of ICR strain (weight: 35-40g) purchased from the Medical Research Institute Boralla, Sri Lanka were used. The animals were kept under standardized animal house conditions (photoperiod: approximately 12h natural light per day; temperature: 28-30 °C; relative humidity: 50-
55%) with free access to tap water and pelleted food (Master Feed Ltd., Colombo, Sri Lanka). All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the society of toxicology in 1999) and rules of the Department of Zoology, Faculty of Science, University of Colombo, for animal experimentation.

**Manufacture of tea samples**
The black tea belonging to the grade of Dust No 1 was manufactured at St. Coombs estate tea factory of the Tea Research Institute, Talawakelle, Sri Lanka, with its own green leaves (1382 m above mean sea level) using the orthodox- rotoevane manufacture technique. Tea samples were packed in triple laminated, aluminum foil bags, (1 kg each) and stored at −20 °C until use. The *C. sinensis* leaves used to manufacture the black tea samples was identified and authenticated by Professor (Mrs.) A.S. Senaviratna, Department of Plant Science, University of Colombo. A voucher specimen (wdr/tspf 200) was deposited at the museum of the Department of Zoology University of Colombo.

**Preparation of Black tea brew (BTB)**
Black tea brew (BTB) was made according to the ISO standards ⁹: adding 2g of black tea to 100 ml of boiling water and brewing for 5 min. This contains 43.7% (w/w) tea solids in water. Based on this data, 1336 mg/ml (equivalent to 24 cups, 1 cup = 170 ml) of BTB in 2 ml for oral administration was made by adding 8g black tea to 15 ml boiling water and brewing for 5 min. 501 mg/ml (equivalent to 9 cups), 167 mg/ml (equivalent to 3 cups) and 84 mg/ml (equivalent to 1.5 cups) concentrations of BTB were then made by diluting appropriately with boiling water.

**Evaluation of antinociceptive activity using tail flick test**
This was performed as described by Langerman et al ¹⁰. Fifty four male rats were randomly divided into 6 equal groups (N = 9/group) and were orally treated in the following manner: group 1 with 2 ml of water; group 2 with 84 mg/ml dose of BTB; group 3 with 167 mg/ml dose of BTB; group 4 with 501 mg/ml dose of BTB; group 5 with 1336 mg/ml dose of BTB; and group 6 with reference drug aspirin (133.5 mg/kg). Two to three hours before treatment and then at hourly intervals for 6h (post treatment) these rats were placed on an enclosed hot plate (Model MK 35A, Maroma Co. Ltd., Tokyo, Japan) at 50 °C and the time taken (reaction time) to lick either hind paw or jump up was recorded as described by Langerman et al ¹⁰. A cut off time of 20 s was used to avoid tissue damage.

**Evaluation of antinociceptive activity using formalin test**
This was performed essentially as described by Farsam et al ¹¹. Fifty four male rats were randomly assigned into 6 equal groups (N = 9/group) and were orally treated in the following manner: group 1 with 2 ml of water; group 2 with 84 mg/ml dose of BTB; group 3 with 167 mg/ml dose of BTB; group 4 with 501 mg/ml dose of BTB; group 5 with 1336 mg/ml dose of BTB; and group 6 with reference drug aspirin (133.5 mg/kg). One hour after administration, each rat was injected with 0.05 ml of 2.5% formalin solution (BDH chemicals, Poole, UK) into subplanter surface of the left hind paw. The rats were then observed for 60 min and the numbers of flinching, lifting and licking and the cumulative time spent on lifting and licking were recorded in two phases: early phase 1-5 min and late phase 15-60 min. The time per lift and lick were then computed.

**Evaluation of antinociceptive activity using acetic acid-induced writhing test**
Forty five mice were randomly divided into 5 equal groups (N = 9/group) and were orally treated in the following manner: group 1 with 2 ml of water; group 2 with 84 mg/ml dose of BTB; group 3 with 167 mg/ml dose of BTB; group 4 with 501 mg/ml dose of BTB; group 5 with 1336 mg/ml dose of BTB; and group 6 with reference drug aspirin (133.5 mg/kg). One hour later, 0.5 ml of 10% acetic acid was injected intraperitoneally to each of these mice and number of writhings (abdominal contractions and stretches) that occurred between 5- 20 min were counted and recorded as described by Ojewole ¹².

**Evaluation of antihyperalgesic activity**
This was performed as described by Richardson et al ¹³. Thirty six male rats were randomly assigned into 4 equal groups (N = 9/group) and were orally treated in the
following manner: group 1 with 2 ml of water; group 2 with 84 mg/ml dose of BTB; group 3 with 167 mg/ml dose of BTB; and group 4 with 501 mg/ml dose of BTB. Immediately afterwards, these rats were subcutaneously injected with 0.05 ml of 1% carrageenan suspension in normal saline into the planter surface of the left hind paw to induce pain. These rats were subjected to hot plate test at hourly intervals for 6h as described previously and the reaction time was determined.

**Investigation of development of tolerance to BTB with chronic administration on hot plate test**

Eighteen rats were randomly divided into two equal group (N = 9/group). One group was orally administered with 2ml of water daily for 43 consecutive days and the other with the 501 mg/ml dose of BTB. These rats were subjected to hot plate test as described previously 1h following treatment on days 1, 15, 29, and 43 of treatment and the reaction time was determined.

**Evaluation of effects of BTB on nervous co-ordination, muscle strength and rectal temperature**

Eighteen rats were randomly divided into two equal group (N = 9/group). One group was orally treated with 2 ml of water and the other with the 501 mg/ml dose of BTB. One hour later, these rats were subjected to Bridge test and Righting reflex test (to investigate its effect on nervous co-ordination), and Bar test (for muscle strength) and their respective latencies were recorded. Immediately following this their rectal temperatures were determined using a digital thermometer (TM -II, Focal Corporation, Tokyo, Japan).

**Investigation of involvement of opioid receptor mediation**

Twelve rats were divided into two groups. Those in-group 1 (N = 6) were subcutaneously injected with 5 mg/kg naloxone (an opioid receptor antagonist) and those in group 2 (N = 6) with isotonic saline. After 45 min, rats in both groups were orally administered with 2 ml of 501 mg/ml dose of BTB. These rats were subjected to the hot plate test before treatment and 1 h after BTB treatment and the reaction time was determined.

**Involvement of dopamine receptor mediation**

Twelve rats were randomly divided into two equal groups. The rats in group 1 (N = 6) were orally administered with 1.5 mg/kg of metachlopramide, a dopamine D2 antagonist, in 1ml of methylcellulose. The rats in group 2 (N = 6) were orally treated with 1 ml of methylcellulose. After 60 min both groups of rats were orally treated with the 501 mg/ml dose of BTB. These rats were subjected to hot plate test before treatment and 1 h after BTB treatment and the reaction time was determined.

Investigation of involvement of muscarinic receptor mediation

Twelve rats were randomly divided into two equal groups. Those in group 1 (N= 6) were intraperitoneally injected with 5 mg/kg of atropine sulphate, a muscarinic receptor antagonist, and those in group 2 (N = 6) with isotonic saline. After 10 min, the rats in both groups were orally administered with the 501 mg/ml dose of BTB. One hour later, these rats were subjected to hot plate test, before treatment and 1 h post treatment with BTB and reaction time was determined.

**Investigation of sedative activity**

Twelve rats were randomly divided into two equal groups. One group (N = 6) was orally treated with the 501 mg/ml dose of BTB and other group (N = 6) treated with 2 ml of water. After 1 h, each rat was placed in the center of the rat hole-board and observed for 7.5 min. The number of rears, number of head dips and locomotary activity were recorded as described by File and Wardill 18. Time per head dip was then computed.

**Evaluation of antihistamine activity**

Eighteen rats were randomly assigned into two equal group (N = 9/ group). The left posterior lateral side of their skin was clearly shaved under aseptic conditions. One group was orally treated with the 501 mg/ml dose of BTB and the other with 2 ml water. After 1 h, 50 µl of 200 µg/ ml of histamine in normal saline was subcutaneously injected to the shaved area of the skin and the area of the wheal formed was determined after 1.5 min.

**Effect on membrane stabilization**

This activity was evaluated using heat-induced haemolysis of rat erythrocytes in vitro as described by Dharmasiri et al 20. Vials containing 20 µl fresh rat blood in 1 ml of phosphate buffered saline were treated in triplicate with the BTB so that the final concentrations of the tea brew in the vials were 2.5, 5, 10 and 20 mg/ml. Fifteen microliter of saline was used as the control. The vials were then incubated for 15 min at 37 ºC followed by 54 ºC for 25 min, centrifuged at 3200 x g for 2 min and the absorbance of the supernatant was measured at 540 nm using a spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan). The percent inhibition of haemolysis with respect to the controls was calculated.

**Evaluation of antioxidant activity (DPPH assay)**

This was done using 750 µl of freshly prepared 20ppm of 1:1-diphenyl-2-picrylhydrazyl (DPPH) solution as described in detail by Abyewickrama et al 21. Briefly, 3 concentrations of BTB (84, 167, 501 mg/ml) types were made, and 750 µl of these samples were added to 750 µl of DPPH solution (in triplicate) and incubated at 30 ºC for 5 min. The absorbance was then measured at 517 nm.
RESULTS

Tail flick test
As shown in Table 1, compared to control, all doses of BTB tested, significantly (P < 0.05) increased (84 mg/ml dose by 95%; 167 mg/ml dose by 25%; 501 mg/ml dose by 21%; 1336 mg/ml dose by 31%) the reaction time in the tail flick test at 6 h post-treatment. This effect was however, not dose-dependent (r² = 0.30; P > 0.05). In addition, moderate but significant (P < 0.05) prolongations of the reaction time was evident with the 84 mg/ml dose at 5th (by 50%), 501 mg/ml dose at 3rd h (by 44%) and 1336 mg/ml dose at 2nd h (by 26%) and 4th h (by 30%) post treatment. On the other hand, aspirin prolonged the reaction time at all time points from the 2nd h up to 6th (2nd h by 12.5%, 3rd h by 13%, 4th h by 20%, 5th h by 31% and 6th h by 57%).

Hot plate test
The results of the hot plate test with male rats are depicted in Table 2. As shown, 501 mg/ml (1st h by 30% and 2nd h by 38%) and 1336 mg/ml (1st h by 79% and 2nd h by 38%) doses of BTB moderately and significantly (P < 0.05) increased the reaction time at 1 and 2 h of post treatment. The reference drug, aspirin significantly (P < 0.05) increased the reaction time upto 4th h (1st h by 71%, 2nd h by 60%, 3rd h by 42% and 4th h by 21%).

Formalin test
Table 3 summarizes the results of the formalin test. As shown, none of the doses of BTB significantly (P > 0.05) altered any of the parameters investigated during the early phase (0-5 min). On the other hand, 501 mg/ml dose (number flinchings by 58%; number of liftings by 34%; number of lickings by 12.5%; time spent on lifting by 70%; time spent on licking by 49%; time per lift by 70% and time per lick by 49%) and the 1336 mg/ml dose (number of flinchings by 58%; number of liftings by 34%; number of lickings by 12.5%; time spent on lifting by 70%; time spent on licking by 49%; time per lift by 70% and time per lick by 49%) significantly (P < 0.05) suppressed the parameters investigated during the late phase (20-60 min). In contrast, aspirin, the reference drugs significantly (P < 0.05) inhibited all the four parameters of this test at the second phase (number of flinchings by 72%; number of liftings by 43%; number of lickings by 30%; time spent on lifting by 13%; time spent on licking by 50%).

Writhing test
As shown in Table 4 only the 501 mg/ml (by 35%) and the 1336 mg/ml (by 37%) doses of BTB significantly (P < 0.05) and markedly impaired the number of writhings. However, this effect was dose dependent (r² = 0.89; P < 0.05). The reference drug, diclofenac significantly (P < 0.05) and profoundly reduced (by 91%) the number of writhings.

Anti-hyperalgesic activity
As shown in Table 5, all the three doses of BTB significantly (P < 0.05) increased the reaction time upto 3h (1st h, 84 mg/ml by 32%; 167 mg/ml by 19%; and 501 mg/ml by 17%; 2nd h, 84 mg/ml by 40%; 167 mg/ml by 22%; 501 mg/ml by 10%; 3rd h, 84 mg/ml by 23%; 167 mg/ml by 19%; and 501 mg/ml by 19%). These effects were inversely dose-related (1st h, r² = -0.83, P < 0.05; 2nd h, r² = -0.77, P < 0.05 and 3rd h r² = -0.75, P < 0.05).

Investigation of development of tolerance
Continuous administration of the 501 mg/ml dose of BTB evoked a significant (P < 0.05) increase in reaction time on days 1 (control versus treatment: 10.90 ± 0.10 versus 12.96 ± 0.10 s) day 15 (control versus treatment: 10.50 ± 0.08 versus 12.60 ± 0.04 s) 29 (control versus treatment: 11.25 ± 0.05 versus 13.98 ± 0.03 s) and 43 (control versus treatment: 11.80 ± 0.05 versus 13.40 ± 0.06 s). Further, the magnitude of the response was essentially similar in all the days tested.

Muscle co-ordination, muscle strength and rectal temperature
The 501 mg/ml dose of BTB did not significantly (P > 0.05) alter the reaction time either in the Bridge test using a spectrophotometer. The percentage of the DPPH radical scavenged by the tea extracts was calculated, and the antioxidant activity was expressed as the Trolox equivalent in μg/l.  

Overt signs of toxicity
All rats used in the tolerance study were closely observed for 2-3 h, after each daily administration of BTB or vehicle for any overt signs of toxicity (salivation, lachrymation, ptosis, stupor, squinted eyes, teeth exposure, writhings convulsions, tremors, yellowing of fur, loss of hair and breathing depressions), stress (erection of fur and exophthalmic), behavioural abnormalities (such as impairment of spontaneous movements, climbing, cleaning of face and autogrooming, and ataxia, rolling and other postural changes) and aversive behaviours (biting and scratching behaviour, licking of tail, paws and penis, intense grooming behaviour or vocalization) and diarrhoea. In addition, the rats were observed for the presence of ‘Straubs’ tail reaction, and food and water intake.

Statistical analysis
Data were expressed as mean ± SEM. Statistical comparison were made using one-way ANOVA with Turkey’s family error post hoc test. Comparison of unpaired data was done by using Mann-Whitney U-test. Dose dependencies were determined by using Pearson’s correlation test. Significance level was set at P< 0.05.
The antioxidant activity of tea samples was evaluated in vitro. As shown, BTB exhibited dose-dependent ($r^2 = 0.83$, $P < 0.05$) antioxidant activity in vitro.

### Overt signs of toxicity

Oral treatment of the 501 mg/ml dose of BTB did not induce signs of overt toxicity, stress or aversive behaviors. Further, the BTB treatment did not produce Straub's tail reaction or suppressed food and water intake.

### DISCUSSION

The results clearly demonstrate, for the first time, that strong tea brew of *C. sinensis* made from Sri Lankan high grown Dust grade No: 1 black tea possesses oral antinociceptive activity as evaluated from tail flick, hot plate, writhing and formalin algesiometric models, which are scientifically validated and widely used tests to evaluate potential antinociceptive agents. The positive results in the first two tests suggest that the BTB is effective against transient phasic pain which is centrally mediated both at spinal and supraspinal levels: the tail flick technique predominately measures spinal reflexes whilst the hot plate test predominately measures supra spinal reflexes $^{22}$. On the other hand, the positive result in the writhing test indicates that the BTB is effective against peripheral and non specific pain of visceral origin $^{23}$, whilst the results of the formalin test indicate its effectiveness against peripheral continuous inflammatory pain (particularly the second phase) $^{24}$.

Overall, these were genuine and primary effects and not secondary manifestations springing from motor deficits (as reflected from bar test and undepressed locomotory activity in the rat hole-board test), nervous incoordination (as judged by righting reflex and Bridge tests) or hypothermia (in terms of rectal temperature). In addition, the results show that the BTB has antihyperalgesic activity (as judged by the results of carrageenan-induced thermal hyperalgesic test and late phase of formalin test). Possessing of antinociception, especially, against continuous inflammatory pain and antihyperalgesic activity of rapid onset by BTB brew without development of tolerance are important features. Continuous inflammatory pain is one of the most common type of pathological pain in clinical practice and persistent pain is known to have a major impact on the quality of life $^{25}$.

The antinoceptive activity of the BTB had a rapid onset and a short duration of action. This is presumably due to rapid absorption and equally rapid degradation and/or equally rapid clearance of the active component(s). Alternatively, the short duration of action may be due to the formation of a metabolite which is antagonist to the production of antinociceptive effect $^{26}$. Having a rapid onset and a short duration of action of antinociception is some times useful in pain management strategies.

Food restriction induces antinociception in rats $^{27}$ but such a mode of action is unlikely, as food was available...
throughout the study period. Further, hypophagia was not apparent. Stress is known to evoke antinociception. Antinociception in this study cannot be attributed to stress, as there were no signs of exophthalmia, fur erection or aversive behaviours. Further, we have previously shown that BTB is anxiolytic. Membrane stabilizers induce antinociception by elevating pain threshold. Such a mechanism is unlikely to be operative here as the BTB failed to suppress heat-induced haemolysis of rat erythrocytes and the early phase of the formalin test. Sedation is known to impair pain and several sedatives possess antinociceptive activity. However, the antinociceptive activity of BTB is unlikely to be mediated via sedation as none of the parameters of the rat hole-board test was suppressed. Antinociception can be mediated via cholinergic and dopaminergic mechanisms. However, antinociception elicited by BTB was not blocked either by muscarinic receptor antagonist, atropine or dopamine receptor antagonist, metochlopramide. Thus, both these mechanisms are unlikely to be operative here. Tea contains caffeine. But, it is doubtful that BTB induced antinociception is mediated via caffeine: although caffeine is incorporated in some compound analgesic preparations and is claimed to enhance the analgesic effect this is not proven conclusively. On the other hand, naloxone, the universal opioid receptor antagonist, curtailed the antinociception induced by BTB indicating that antinociception is mediated via opioid mechanisms. Importantly, the opioid receptor of rat is identical in structure and pharmacology to human receptors and hence this result is likely to be applicable to man. In this study, there was a lack of typical μ-opioid receptor mediated side effects such as breathing depression, elicitation of Straubs tail reaction and sedation possibly suggesting binding and activation of δ- and κ-opioid receptors in mediating the antinociceptive action. The diminished tail flick response compared to hot plate response with the treatment of BTB may provide additional support for this notion as μ-opioid receptors are the preeminate type in the rat spinal cord. The stimulation of opioid receptors by tea could be due to direct agnostic activities of opioidiometric constituents in the brew and/or due to increase release of endogenous opioid peptides. After all, tea brew contains a variety of phyto constituents. Further, experiments are obviously needed for the elucidation of these potential mechanisms. BTB suppressed the numbers of paw lickings, flinchings or liftings and, licking and lifting durations in the late phase of the formalin test. Pain induced in the late phase of the formalin test is due to release of inflammatory mediators such as prostaglandins, histamine, serotonin or bradykinin at the site of injection. BTB has antihistamine (wheat test) and PG synthesis inhibiting activity and thus could account for the inhibition of the late phase of the formalin test. Opioids can also inhibit the late phase of this test. This mode of action is also likely to be responsible as nalaxone blocked the BTB induced antinociception in the hot plate test. In addition, phenolic continuants of tea could also block the late phase of formalin test. Prostaglandins are involved in the mediation of pain and prostaglandin synthesis blockers are widely used as analgesics. BTB is shown to possess prostaglandin synthesis blocking activity and inhibition of writhing in the writhing test in this study also supports this finding. Therefore this mode of action too could play a substantial role inducing antinociception in this study. Free radicals are now implicated with pain and some plant antioxidants have pain-alleviating properties. Dust grade tea had antioxidant activity (by DPPH assay) in agreement with others and this action could also play an important role in inducing antinociception. This may be attributed to flavonols of the tea. Morphine, a well-known opioid agonist was also proven to possess antioxidant properties. Nitric oxide is also now linked with pain. Black tea is known to reduce nitric oxide production. Therefore, possibility exists that this mechanism could, at least, play an auxiliary role in inducing antinociception in this study. Interestingly even with daily chronic administration of strong BTB, there were no overt signs of toxicity. Other studies have shown long term administration of strong BTB does not produce renal (in terms of serum creatinine, urea and Na⁺, K⁺ level) and hepatic (in terms of SGOT and SGP and serum protein) toxicity. One of the serious side effects of analgesics acting via inhibition of prostaglandin synthesis is gastric haemorrhage due to induction of gastric lesions. However, BTB in addition to its pain relieving action is shown to have gastroprotective and gastric ulcer healing activity. This is obviously a plus point for BTB as a antinociceptive agent. In conclusion, this study, shows for the first time, that strong black tea can act as safe orally active, short acting moderate antinociceptive agent actively via multiple mechanisms. Consumption of strong BTB may be useful to relieve day-today body aches and pains. Further, studies are however warranted before firm recommendations are made.
ACKNOWLEDGEMENT
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REFERENCES
Table 1: Effect of oral administration of Sri Lankan high grown Dust grade No 1 tea brew (Camellia sinensis) on the reaction time of rats in the tail flick test (mean ±SEM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Reaction time (sec)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre treatment</td>
<td>Post treatment</td>
</tr>
<tr>
<td></td>
<td>1st hour</td>
<td>2nd hour</td>
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<td>Control 2 ml water</td>
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<td>BTB 84 mg/ml</td>
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<td>167 mg/ml</td>
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<td>501 mg/ml</td>
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<td>1336 mg/ml</td>
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<td>Reference drug Aspirin 133.5 mg/ml</td>
<td>1.45 ± 0.20</td>
<td>2.65 ± 0.50</td>
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* P< 0.05 compared to control (ANOVA with Turkey’s family error post hoc test)

Table 2: Effect of oral administration of Sri Lankan high grown Dust grade No 1 black tea brew (Camellia sinensis) on the reaction time of rats in the hot plate test (mean ±SEM)

<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Dose</td>
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<td>1336 mg/ml</td>
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<td>Aspirin 133.5 mg/ml</td>
<td>11.90±1.10</td>
<td>17.45 ± 2.26*</td>
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*P< 0.05 compared to control (ANOVA with Turkey’s family error post hoc test); BTB= Black tea brew
<table>
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<tr>
<th>Treatment</th>
<th>Early phase (0-5min)</th>
<th>Late phase (20-60 min)</th>
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<tr>
<td></td>
<td>Number of flinching</td>
<td>Number of lifting</td>
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<tr>
<td>Control</td>
<td>58.0 ± 0.85</td>
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<td>BTB</td>
<td>84 mg/ml</td>
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<td>501 mg/ml</td>
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<td>Reference Drug Aspirin 133.5 mg/ml</td>
<td>58.9 ± 0.25</td>
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*P< 0.05 compared to control (ANOVA with Turkey’s family error post hoc test); BTB= Black tea brew

<table>
<thead>
<tr>
<th>Treatment</th>
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<td>Control</td>
<td>54.4 ± 0.50</td>
</tr>
<tr>
<td>BTB</td>
<td>84 mg/ml</td>
</tr>
<tr>
<td></td>
<td>167 mg/ml</td>
</tr>
<tr>
<td></td>
<td>501 mg/ml</td>
</tr>
<tr>
<td></td>
<td>1336 mg/ml</td>
</tr>
<tr>
<td>Reference drug</td>
<td>Diclofenec (100mg/ml)</td>
</tr>
</tbody>
</table>

*P< 0.05 compared to control (ANOVA with Turkey’s family error post hoc test); BTB= Black tea brew

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reaction time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st hour</td>
</tr>
<tr>
<td>Control (water)</td>
<td>11.35±0.12</td>
</tr>
<tr>
<td>84 mg/ml</td>
<td>11.74±10.20</td>
</tr>
<tr>
<td>167 mg/ml</td>
<td>12.01±0.12</td>
</tr>
<tr>
<td>501 mg/ml</td>
<td>11.24±0.43</td>
</tr>
</tbody>
</table>

*P< 0.05 compared to control (ANOVA with Turkey’s family error post hoc test); BTB= Black tea brew
Table 6: *In vitro* antioxidant activity of Sri Lankan high grown Dust grade No 1 black brew of *Camellia sinensis* as determined by DPPH assay (means ± SEM).

<table>
<thead>
<tr>
<th>Tea sample</th>
<th>Antioxidant activity (Trolox equivalents µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Tea Brew</td>
<td></td>
</tr>
<tr>
<td>Low concentration (83 mg/ml)</td>
<td>2985 ± 6.0</td>
</tr>
<tr>
<td>Mid concentration (169 mg/ml)</td>
<td>3572 ± 86.5</td>
</tr>
<tr>
<td>High concentration (501 mg/ml)</td>
<td>3923 ± 6.5</td>
</tr>
</tbody>
</table>

DPPH = 1,1-diphenyl-2-picrylhydrazyl

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