Antidepressant and Antibacterial Activities of Camellia sinensis (White Tea)

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Abstract

The present study was designed to investigate the antidepressant potential of ethanolic extract of leaves and buds of *Camellia sinensis* (White tea) at 300 mg/kg doses in mice and antibacterial potential by disc diffusion method. Antidepressant activity of the ethanolic extract of white tea (EEWT) was assessed by using hole cross test, open field test and thiopental induced sleeping time test in swiss albino mice. The efficacy of extract (300mg/kg) was compared with standard anxiolytic drugs diazepam (1mg/kg) orally. The extract increased the locomotor activity of mice in open field and hole-cross test significantly ($p<0.05$). Moreover, the extract significantly ($p<0.05$) maximized onset of sleep and minimized the duration of sleeping time when administered with thiopental sodium. On the other hand, to determine antibacterial activities, the extract was tested against two Gram positive and five Gram negative bacteria at three concentrations (200, 300, 500µg/disc) through disc diffusion method. The antibacterial activities of ethanolic extract of leaves and buds of *Camellia sinensis* (white tea) were screened against various pathogenic bacteria such as *Streptococcus agalactiae, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Shigella sonnei, Shigella boydii* by 'disc diffusion method'. The zone of inhibition ranges from 6 to 11 mm and the highest zone of inhibition is 11mm which was found for *Shigella sonnei* at 500µg/disc. Ethanolic extract of Leaves and buds of *Camellia sinensis* (white tea) exhibited moderate to less activity against some organisms tested compared with the standard antibiotic "Kanamycin". These findings demonstrate that the extract of leaves and buds of *Camellia sinensis* have significant antidepressant activity and moderate antibacterial activity.

Keywords
Camellia sinensis, White tea, antidepressant, antibacterial, hole-cross, open field, thiopental sodium, disc diffusion.

Introduction

Camellia sinensis is the species of plant whose leaves and leaf buds are used to produce the popular beverage tea. It is of the genus Camellia, a genus of flowering plants in the family Theaceae. White tea, green tea, oolong, pu-erh tea and black tea are all harvested from this species, but are processed differently to attain different levels of oxidation. Kukicha (twig tea) is also harvested from Camellia sinensis, but uses twigs and stems rather than leaves.
White tea is the least processed form of tea, made of beautiful silver buds and select leaves which have been steamed and dried. Because of its minimal processing, white tea contains more nutrients than its black or green cousins, making it the mightiest of the teas, the ultimate Health Tea. The herbs white tea (Camellia sinensis) was recently reported to have high polyphenolic contents and to exhibit high activities in antioxidant assays, along with potential anti-ageing activity via inhibition of collagenase and elastase (Thring et al., 2009). These herbs are often included in skin care products and are usually advertised for their astringent and antioxidant properties. In the scientific literature, white tea is reported for topical treatment of skin disorders. White tea has antiseptic and antioxidant (Van Wyk et al., 2004) and anti-inflammatory properties (Thring et al., 2011). It also exhibits the excellent cytotoxic and thrombolytic properties (Rashid et al., 2013). The aims of this study were to further explore the antidepressant and antibacterial activity of the ethanolic plant extract of white tea.

Materials and Methods

Plant material

The leaves and silver buds of *Camellia Sinensis* were collected from Botanical garden of Jahangirnagar University, Savar, Bangladesh in 2013 and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Extracts preparation

The collected plant were washed thoroughly water and air dried for a week at 35-40oC & pulverized in electric grinder. The powder obtained was successively extracted in ethanol (55-60oC). The extracts were made to dry by using rotary evaporator under reduced pressure.

Animals

Swiss albino mice having weight 25-30 gm were collected from the renowned animal lab of Jahangirnagar University (JU), Savar, Bangladesh. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0±2.0°C and 12 h light: dark cycle) and acclimatized for 7 days. The animals were fed with standard diet and water. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Test Organisms

The bacterial strains used for the experiment were collected as pure cultures from the well equipped microbiology lab of Department of Pharmacy, International Islamic University Chittagong.

Antidepressant Activity
Hole Cross Test

The test was observed by the method described by Takagi *et al.*, (1971) for screening sedative activity in mice. The animals were divided into three groups- control, positive control and test. The test groups received methanolic extract of *C. sinensis* at the doses of 300 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passages of a mouse through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral treatment with test drugs. Diazepam was used in the positive control group as reference standard at the dose of 1 mg/kg (i.p).

Open Field Test
The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta *et al*., (1971). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the mice was counted for 3 min, on 0, 30, 60 and 120 min during the study period.

Thiopental sodium induced sleeping time test
The experiment was conducted following the method described by Ferrini *et al* (1974). The animals were randomly divided into three groups consisting of five mice each. The test groups received methanol extract from the leaves of *C. sinensis* at dose 300 mg/kg (p.o) body weight while the standard group was treated with diazepam (1 mg/kg, p.o) and control group with vehicle (1% Tween 80 in water). Twenty minutes later, thiopental sodium (40 mg/kg, i.p) were administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex.

Antibacterial assay
In vitro antibacterial screening is generally performed by disc diffusion method (Bauer et al., 1966; Barry et al., 1986; Rios et al., 1988) for primary selection of the compounds as therapeutic agent. Disc diffusion method is equally suited to screening of antibiotics or the products of plant evaluation (McGowan, 2001) and is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. In this method the compounds are applied to the agar medium by using paper discs (Phalguni et al., 2000). The method is essentially a qualitative or semi quantitative test which allows classification of microorganisms as susceptible, intermediate or resistance to the test materials as well as bacteriostatic or bactericidal activity of a compound (Reiner, 1982). The diameters of the zone of inhibition produced by the compounds at the concentration 200, 300 & 500 µg /disc were compared with the standard antibiotic (Kanamycin, 30µg/disc). The experiments were performed at four times to minimize the error.

Statistical analysis

Data are expressed as mean ± STD and were analyzed statistically by one-way ANOVA procedures, followed by using Turkey's test. A difference was considered significant at $p<0.05$.

Results

Antidepressant activity

Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 0 to 120 min (Table 1). In the hole cross test, the extracts showed an increase in locomotion in the test animals from the second observation period as evident by the augmentation of number of hole crossed by the treated mice compared to the control group. The result was comparable to the reference drug diazepam and was statistically significant ($p< 0.05$).

Table 1. Antidepressant activity of ethanolic extract of white tea (EEWT) on hole cross test in mice

Group	**Treatment**	**Dose, Route**	**Numbers of movements**				
			0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	1% tween 80 in water	10 mg/kg, p.o	18.67± 0.882	16.33± 0.67	14.67± 1.202	15.67± 1.202	15.67±0. 882
Standard	Diazepam	1 mg/kg, p.o	13.00± 1.155*	6.00± 1.528*	4.33± 0.33*	3.67± 0.67*	1.33± 0.33*
Test	EEWT	300 mg/kg, p.o	10.67± 0.67*	22.00± 1.155*	24.00± 2.082*	20.33± 2.082*	18.33±0. 33*
All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Turkey's test. *P<0.05, significant compared to control.							

Open field test

In the open field test, the number of squares traveled by the mice was enhanced significantly in the test group from 2nd observation compared to the control group. This increase number of squares traveled by the mice indicates the antidepressant activity of extract.

Table 1. Antidepressant activity of ethanolic extract of white tea (EEWT) on open field test in mice

Group	**Treatment**	**Dose, Route**	**Numbers of movements**				
			0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	1% tween 80 in water	10 mg/kg, p.o	68.00± 1.528	65.67 ±0.882	49.33± 1.856	46.33± 1.202	48.33± 2.728
Standard	Diazepam	1 mg/kg, p.o	66.33± 4.256	54.67 ±4.33	34.00± 2.646*	19.67± 0.882*	17.67± 1.33*
Test	EEWT	300 mg/kg, p.o	76.00± 3.786	169.67± 1.453*	146.33 ±4.807*	123.3± 5.548*	77.33± 5.608*
All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Turkey's test. *P<0.05, significant compared to control.							

Thiopental sodium induced sleeping time test

In the thiopental sodium induced sleeping time test, the test group treated with the extract at 300 mg/kg showed significant ($p<0.05$) increase in onset of action and decreased the duration of sleep which means that the extract may have antidepressant effect. The extract showed higher onset than control and diazepam and lower duration of sleep than control

Table 3. Antidepressant activity of ethanolic extract of white tea (EEWT) on on thiopental sodium induced sleeping time test in mice

Group	**Treatment**	**Dose, Route**	**Onset of sleep (min)**	**Duration of sleep (min)**
Control	1% tween 80 in water	10 mg/kg, p.o	7.33± 1.202	46.33± 0.67
Standard	Diazepam	1.00 mg/kg, p.o	2.33±0.33*	147.33± 6.566*
Test	EEWT	300 mg/kg, p.o	17.67±1.202*	23.33± 3.528*
All values are expressed as mean ± SEM (n=5); One way Analysis of Variance (ANOVA) followed by Turkey's test. *P<0.05, significant compared to control.				

Antibacterial Assay

Antibacterial activities of the extract were tested against seven pathogenic bacteria and were compared with the standard antibiotic Kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) showed in **table 4.**

Table 4 Antibacterial activity of ethanolic extract of white tea (EEWT).

SL. No.	**Name of the bacteria**	**Sample Extracts (Zone of inhibition in mm)**			**Standard***
		200 µg/disc	**300 µg/disc**	**500 µg/disc**	**30 µg/disc**
B01	*Streptococcus agalactiae*	Nil	Nil	Nil	32
B02	*Bacillus cereus*	Nil	Nil	8	35
B03	*Pseudomonas aeruginosa*	Nil	Nil	10	33
B04	*Proteus mirabilis*	Nil	Nil	Nil	35
B05	*Escherichia coli*	Nil	6.5	9	30
B06	*Shigella sonnei*	6	9	11	31
B07	*Shigella boydii*	Nil	Nil	6	29
***Standard: Kanamycin Nil: Not susceptible**					

Discussion

The present study demonstrated that the administration of 300 mg per kg body weight dose of methanol extract from C. sinensis leaves and silver buds shows antidepressant properties. The dose dependently reduced sleep induced by thiopental suggesting that the extract of white tea did not possess a sleep inducing property. "Thiopental" basically a hypnotic agent, given at appropriate dose, induced hypnosis by potentiating GABA mediated postsynaptic inhibition through allosteric modification of GABAA receptors. Substances which possess antidepressant activity either prolong the time for onset of sleep or decrease the duration of sleep or both (Nyeem et al., 2006; Raquibul Hasan et al., 2009). In addition, the study on locomotor activity, as measured by hole cross and open field tests, showed that both doses of ethanolic extract from the leaves and silver buds of *C. sinensis* increased the frequency and the amplitude of movements. Since locomotor activity is a measure of the level of excitability of the CNS (Mansur et al., 1980), this increase in spontaneous motor activity could be attributed to the antidepressant effect of the plant extracts (Rakotonirina et al., 2001). The dose of 300 mg/kg body weight significantly raised the locomotion in mice. The locomotor activity raising effect was evident at the 2nd observation (30 min) and continued up to the 5th observation period (120 min).

During screening for antimicrobial activity, the EEWT exhibited moderate antimicrobial activity (zone of inhibition = 6.0-11.0 mm) against the test organisms. The crude ethanolic extract of white tea exhibited moderate antimicrobial activity against *Sigella sonnei* (11.0 mm), *Pseudomonas aeroginosa* (10.0 mm), *Escherichia colli* (9.0 mm) and *Bacillus cereus (8.0 mm)* at 500 µg/disc.

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Conflict of interest

The Authors have declared that there no conflict of competing interest.

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