



NEURO PHARMACOLOGICAL STUDY OF LEAVES OF *CAMELLIA SINENSIS*

MANGAL ASHISH*, JAIN VINAY, JAT R.C., BHARADWAJ SUDHIR AND JAIN SUMAN

ShriRam College of Pharmacy Banmore, Morena (M.P.) Email: sarapharma200389@yahoo.com, ashishmangal.200200@yahoo.com

Received: 18 April 2010, Revised and Accepted: 04 May 2010

ABSTRACT

Camellia sinensis commonly known as green tea, is mainly cultivated in tropical and sub tropical climate. To the best of our knowledge the plant is so far reported only for memory enhancing properties. In this content the neuropharmacological study of *camellia sinensis* for anxiolytic activity was assessed by hole board test, Y maze test, social interaction test, foot shock induced aggression test for aqueous extract of *camellia sinensis*. Two doses of 200 ml/ Kg and 400 ml/ kg of aqueous extract of *camellia sinensis* were used. The result from this study strongly suggests that *camellia sinensis* possess varied effects on the CNS including anxiolytic, activity.

Keywords: *Camellia sinensis*, anxiolytic activity, Y maze test, social interaction test, foot shock induced aggression test.

INTRODUCTION

The Indian system of medicines, Viz Ayurveda, Siddha, Unani and Homeopathic system predominantly use plant based raw materials and most of their preparations and formulations. Herbal medicines are becoming more and more popular nowadays. Among the entire flora, 35,000 to 70,000 species have been used for medicinal purposes¹. In India, of the 17,000 species of higher plants, 7500 are known for medicinal uses. This is the highest proportion of medicinal plants known for their medical purposes in any country of the world for the existing flora of that respective country^{2,3}. Demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at desirable price and sometimes the only source of health care available to the poor.

Mental health problems currently are said to constitute about eight percent of the global burden of disease and more than 15 percent of adults in developing societies are estimated to suffer from mental illness. According to the new concept of measuring disability called Disability Adjusted Life Years (DALY), mental disorders constitute a significant part of total disability adjusted life years (8.1%), more than the disability caused by several well-recognized disorders such as cancer (5.8%) and heart diseases (4.4%). Mental disorders may not produce high mortality rates (except for a proportion of suicides) but do give rise to high morbidity which implies debility or disability. The seriousness of the problem in India is indicated by the fact that the estimated overall prevalence rates of mental illness vary from 9.5/1000 to 102.5/1000. It is further estimated that nearly thirty million suffer from mental illnesses every year and that 175,000 new cases are added every year.

Depression and anxiety are the most frequent mental disorders. More than 20% of the adult population suffers from these conditions at some time during their life. The World Health Organization (WHO, 1999) predicts that depression will become the second leading cause of premature death or disability worldwide by the year 2020. Approximately two-thirds of the anxious or depressed patients respond to the currently available treatments but the magnitude of improvement is still disappointing. Then, the medical need for newer, better-tolerated and more efficacious treatments remains high.

Since ancient time the herbal medicines are effective in the treatment of various ailments. Many plants have folklore claim in the treatment of several dreadful diseases but they are not scientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern medicine⁴.

Camellia sinensis traditionally used to treat asthma (functioning as a bronchodilator), angina pectoris, peripheral vascular disease and coronary artery disease. It has been most used as a stimulant, or as an astringent lotion, which may be used as a gargle or injection. An infusion of tea leaves was once used as a remedy for insect bites. An

infusion of tea has been used for some digestive problems and to reduce sweating in fevers. In Tamil Nadu, tea leaves have been used homeopathically for mania, paralysis, nervousness, neuralgia and sleeplessness⁵.

The literature, revealed that the CNS studies on aqueous extract of *Camellia Sinensis* are sparse, there are reports of *Camellia Sinensis* on protection against cognitive impairment and no detailed study was conducted for its central nervous system effects. Based on the above facts that no thorough scientific study has been carried out in *Camellia Sinensis* regarding its effect of central nervous system the present study is aimed to evaluate the neuropharmacological profile of *Camellia Sinensis*.

MATERIAL AND METHOD

Plant collection and authentication

The plant material of *Camellia Sinensis* leaf used for the investigation was collected from ooty in the month of July. The plant was identified and authenticated from National Institute of herbal science, Chennai-600045.

Extraction

The freshly collected leaves of this plant were shade dried and coarsely powdered. The powder was passed through 40-mesh sieve then, 2 g of tea [*Camellia sinensis* var *assamica* (Theaceae)] was soaked in 10 ml of boiling water. After 5 min, it was filtered. And this aqueous extract of *Camellia sinensis* (AECS) was administered to animal.

Experimental animals

Adult Wistar rats (150-200 gms) and mice (20-30 gms) of both sexes were used in the pharmacological studies. The inbred animals were taken from the animal house in Vel's college of pharmacy, Pallavaram, Chennai -117. The animals were maintained in well-ventilated room temperature with natural 12h \pm 1h day-night cycle in the propylene cages. They were fed balanced rodent pellet diet during experimental period. The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature. The experimental protocol was proved by the Institutional Animal Ethics Committee IAEC Ref No: 290/CPCSEA/PHARMACOL-13/08. Dt.6/9/2008

Experimental design

The present study is designed with the following models in order to assess anxiolytic and Anti-depressant activity of *Camellia Sinensis*

Assessment of anxiolytic activity

1. Hole board test
2. Y- maze test

3. Social interaction test
4. Foot shock induced aggression

Assessment of anxiolytic activity

Treatment Schedule

The mice and rat were divided into four groups consisting of 6 per group and used for the novel environment.

- Group 1: Control (mice were orally administered with drinking 1 water for 5 days)
- Group 2: Mice and rats were orally administered AECS at a dose of 200 mg/kg (1 ml/kg) for 5 days
- Group 3: Mice and rats were orally administered AECS at a dose of 400 mg/kg (2 ml/kg) for 5 days
- Group 4: Standard (Diazepam 4 mg/kg. was administered i.p. 30 min. before experiment)

Hole board test

The Hole Board Apparatus consisted of wooden box (40X 40X 25 cm) with 16 holes (diameter, 3cm) evenly distributed in the floor. The hole board was elevated to the height of 25 cm. The animals were administered the drug 1hr before placing on the apparatus and the time spent in center during 5 min period was recorded⁶.

Y-maze test

Mice were placed individually in symmetrically Y-shaped run way (33x38x13 cm) for 3 min; the number of times a mouse entered in the arm of the maze with all four feet was counted as a single entry and used for the comparison of control and drug treated groups⁷.

Social interaction test

In this test, the amount of time a pair of mice spent socially interacting with one another is thought to reflect the level of anxiety in these subjects. The duration of social interaction decreases with increased anxiety. Mice were placed individually in the test arena for 7-min familiarization session on two consecutive days. During the test day, two randomly selected mice were administered the vehicle, extract, standard drugs and placed in adjacent cages in the waiting

area of the test room. One hr later, they were introduced together into the center of the test arena. Social interaction was observed remotely for 7 min. The number of occurrences of social interactions (sniffing, following, and grooming the partner) and time spent in them were scored⁸.

Foot shock-induced aggression (FSIA)

All the vehicle and extracts were administered, 1hr before the start of the experiment. A pair of male mice was placed in a box with a grid floor consisting of steel rods with a distance of 6 mm. The fighting behavior consisted of vocalization, leaping, running, rearing, and facing each other with some attempts to attack by biting. Six pairs of mice were used for each treatment⁹.

RESULTS

Hole board test

The AECS at both dose level showed a significant ($P<0.001$) increase in time spent in centre when compared to control. The results were comparable with that of standard. Results were shown in *Table 1*.

Y-maze test

In Y-maze test AECS at both dose level showed significant decrease ($P<0.001$) in number of entries when compared to control. The results were comparable with that of standard. The results were shown in *Table 2*.

Social interaction test

In this test, a significant reduction in the number of non-interaction time was observed with high dose level but not at lower dose when compared to the control. The results were comparable with that of standard. Results were shown in *Table 3*.

Foot shock induced aggression

The AECS at both dose levels showed a significant decrease in the number of fighting bouts with increase in the dose when compared to control. The results were comparable with that of standard. Results were shown in *Table 4*.

Table 1: Effect of AECS (Aqueous extract of *Camellia sinensis*) on hole board apparatus

S. No.	Treatment	Time spent in the centre (sec)
1	Vehicle (water)	24.16±0.94
2	AECS (200mg/kg for 5 days p.o.)	69.50±0.99**
3	AECS (400mg/kg for 5 days p.o.)	87.66±1.76**
4	Standard (Diazepam 4mg/kg i.p.)	124.86±2.30**

Statistical significance test was done by ANOVA followed by Dunnet's't' test, ** $P<0.001$ compared to vehicle treated group, Values are Mean ± SEM of 6 animals per group., AECS- Aqueous extract of *Camellia sinensis*

Table 2: Effect of AECS (Aqueous extract of *Camellia sinensis*) on Y-maze apparatus

S. No	Treatment	No. of entries
1	Vehicle (water)	12.0±0.89
2.	AECS (200mg/kg for 5 days p.o.)	6.16±0.60**
3.	AECS (400mg/kg for 5 days p.o.)	3.66±0.66**
4.	Standard (Diazepam 4mg/kg i.p.)	1.66±0.42**

Statistical significance test was done by ANOVA followed by Dunnet's't' test, ** $P<0.001$ compared to vehicle treated group, Values are Mean ± SEM of 6 animals per group., AECS- Aqueous extract of *Camellia sinensis*

Table 3: Effect of AECS (Aqueous extract of *Camellia sinensis*) on social interaction test

S. No.	Treatment	SI	SI time	Non-int. time
1	Vehicle (water)	34.8±2.3	206.9±16.3	207.5±4.2
2	AECS (200mg/kg for 5 days p.o.)	29.8±0.5	209.5±19.1	206.0±19.3 ^{NS}
3	AECS (400mg/kg for 5 days p.o.)	24.3±0.3	201.6±16.5	190.7±16.6*
4	Diazepam (4mg/kg i.p.)	21.2 ± 0.2	180.3 ±15.7	172.6±15.68*

Statistical significance test was done by ANOVA followed by Dunnet's't' test, * $P<0.05$ compared with vehicle treated group, NS-Non significant, Values are Mean ± SEM of 6 animals per group, SI – number of social interactions, SI time – time spent on social interactions., AECS: Aqueous extract of *Camellia sinensis*

Table 4: Effect of AECS (Aqueous extract of *Camellia sinensis*) on Foot shock induced Aggression

S. No.	Treatment	Total No. of fights
1	Vehicle (water)	16.33±3.05
2.	AECS (200mg/kg for 5 days p.o.)	12.50±2.42*
3.	AECS (400mg/kg for 5 days p.o.)	9.0±2.48*
4.	Standard (Diazepam 4mg/kg i.p.)	4.66±1.57**

Statistical significance test was done by ANOVA followed by Dunnet's 't' test, * $P < 0.05$, ** $P < 0.001$, compared to vehicle treated group, Values are Mean \pm SEM of 6 animals per group, AECS: Aqueous extract of *Camellia sinensis*

DISCUSSION

Anxiety, depression, and mental health problems in general and senile neurological disorders in particular, are widely prevalent in modern fast-paced life with a multitude of stressful conditions. While *Camellia Sinensis* produced restriction of movements in animals during the routine screening studies. Few of neuropharmacological activities were reported earlier.

In Hole Board Test AECS showed significant increase in time spent in centre indicating anxiolytic effect.

In Y-Maze Test AECS showed significant decrease in number of entries which indicates anxiolytic effect.

In Social interaction studies an unfamiliar and brightly lit environment, the normal social interaction of rats is suppressed. Anxiolytics counteract this suppression. The rat social interaction model has been used by various authors to characterise the potential anxiolytic effects. Our extract has also showed anxiolytic effect¹⁰.

The reduced number of fighting bouts indicated an anxiolytic activity in foot shock induced aggression¹¹. Not only anxiolytics, but also other classes of drugs such as sedative (meprobamate and Phenobarbital), neuroleptics (perphenazine), and analgesic (methadone), were found to be active in this test.

Thus the AECS possess a significant anxiolytic effect.

REFERENCES

- Edwin E., Sheeja E., Mishra N., Thakre V., and Sudha V. 2005. Herbal medicines: the ups and down. The Ind. Pharmacist. 6, 38-44.
- Chandra PK, Pitamber PD., and Bikram SS., (2006) Developing the medicinal plants sector in northern India: challenges and opportunities. J. Ethnobiol. Ethnomed. 2, 32 doi:10.1186/1746-42692.
- Edwin, E., Joshi, SB. and Jain, DC (2007) A brief review on few indian medicinal plants. Int. J. Green. Pharm. 1 (3-4), 2-12.
- Mental illness. National Association for Mental Health. Available on www.mind.org.uk. Dated on 04-8-2008.
- Vitthal V. Chopade 1, Atul A. Phatak 1, Aman B. Upaganlawar, Anil A, Tankar. Green tea (*Camellia sinensis*): Chemistry, Traditional, Medicinal uses and its pharmacological activities- A review *Phcog Rev:Plant Review*. 2008 Jan- Jun; 2(3), 157-162.
- Clark G, Koster AG, Person DW. Exploratory behaviour in chronic disulfoton poisoning in mice. *Psychopharmacologia (berl)*. 1997; 20:169-171.
- Kulkarni SK. Hand book of experimental pharmacology. 3rd Edn. Vallabh prakashan, New Delhi, India. 2005:59-61.
- Sakina MR, Dandiya PC. A psycho-neuropharmacological profile of *Centella asiatica*. *Fitoterapia*. 1990; 45 (4), 291-296.
- Tedeschi RE, Tedeschi DH, Mucha A, Cook L, Mattis PA, Fellows EJ. Effects of various centrally acting drugs on fighting behaviour of mice. *J Pharmacol Exp. Ther*. 1959; 125:28-34.
- Sakina MR, Dandiya PC, A psycho-neuropharmacological profile of *Centella asiatica*. *Fitoterapia*. 1990; 45 (4): 291-296.
- Turner RA. Screening procedures in pharmacology. *New York. Academic Press*. 1978; 28:154-158.
- Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice. A primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Therapie*. 1977; 229:327-336.
- Steru L, Chemat R, Thierry b, Simon P. The tail suspension test. A new method for screening antidepressants in mice. *Psychopharmacol*. 1985; 85:367-370.