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Anxiolytic Activity Of *Camellia Sinensis* Extract

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Anxiety is defined as an exaggerated feeling of apprehension, uncertainty and fear. Camellia sinensis, popularly known as Green Tea is mainly cultivated in topical and subtopical climate. Its extract contains polyphenols, epigallocatechingallate. Since its antioxidant activity is claimed, we explored Camellia sinensis extract (100, 300, 500mg/kg p.o.) for its anxiolytic activity on albino mice using Elevated Plus Maze, Mirrored Chamber apparatus and Light-Dark model. In the EPM, CS extract showed an anxiolytic effect by increasing time spent and number of entries in open arms. In mirrored chamber apparatus, anti-anxiety effect was confirmed by decreased latency time, increased no. of entries and increased amt. of time spent in mirrored chamber. In Light-Dark Model, extract showed anxiolytic effect by increasing no. of entries and time spent in light compartment. Thus, we can conclude that Camellia sinensis extract possess significant anxiolytic activity and can be used as potential alternative to benzodiazepines.

Keywords: Anxiety, *Camellia sinensis*, Elevated Plus Maze, Mirrored Chamber apparatus, Light-Dark model

Introduction:

Anxiety is defined as an exaggerated feeling of apprehension, uncertainty and fear. It's an unpleasant state of tension with an anticipation of imminent danger [1]. Anxiety is an emotion that allows an individual to prepare for, or respond to, changes in the environment. However, the emotion is expressed inappropriately and impairs their lives causing consistent distress and disability.

Up to 40% of individuals will experience an anxiety disorder in their lifetime and up to 5% will have recurrent or chronic anxiety disorders [2]. Excessive anxiety can weaken and damage the quality of life. [3]

Causes of anxiety disorders: [4]

Heredity/Genetic factors
Brain chemistry
Personality
Life experiences
Stress overload/Lifestyle factors
Thought patterns

The major thrusts of current work dealing with anxiety disorders have centered on GABA mechanisms, serotonergic system, noradrenergic mechanism and neuropeptides. New evidences suggest role for adenosine and cholecystokinin in the development of anxiety. Drug interactions with these neurotransmitters also may have anxiolytic effects [5].

Currently, the most widely prescribed medications for anxiety disorders are benzodiazepines. However, the clinical uses of benzodiazepines are limited by their side-effects such as psychomotor impairment, potentiation of other central depressant drugs and dependence liability. Therefore, the development of new medications possessing anxiolytic effect without the complications of benzodiazepines would be of great importance in the treatment of anxiety-related disorders. Medicinal plants are a good source to find new remedies for these disorders [6].

Camellia sinensis has been used traditionally for cure of various ailments like asthma, coronary artery disease, angina pectoris, peripheral vascular disease mainly contributed to its antioxidant activity [7]. But its use in treatment of anxiety is lacking. So we carried out the study to evaluate antianxiety activity of *Camellia sinensis* in anxiety.

Materials & Methods:

Collection of plant extracts:

Camellia sinensis extract (40%) and was obtained as gift sample from Natural Remedies Pvt. Ltd., Bangalore, India.

Drug:

Diazepam hydrochloride (2 mg/kg) was used as reference drug [3]. All solutions were prepared freshly on test days and administered intraperitoneally.

Animals:

Albino Male mice weighing 20-30 g were procured from Animal House, Anuradha College of Pharmacy Chikhli (Dist-Buldana) (CPCSEA Reg. No. 751/03/abc/CPCSEA. Date: 3 March, 2003) and were used in this study. The mice were housed in environmentally control room with a 12-h light/dark cycle. 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. Animals were naive to experimental conditions. All experiments were carried out in a quiet room between 9:00 a.m. and 2:00 p.m.

Elevated plus maze model of anxiety:[8]

The EPM apparatus consisted of two open arms (16×5cm) and two closed arms (16×5×12cm) emanating from a common central platform (5×5cm). The two pairs of identical arms were opposite to each other. The entire apparatus was elevated to 25cm above the floor level. The animals received the treatment as per the schedule, 1hr before the start of the session. At the beginning of session, a mouse was placed at the centre of the maze, its head facing the open arm. Number of entries and the time spent in closed and open arms were recorded during a 5-min. observation period. An entry was defined as presence of all four paws in the arm.

Mirrored chamber apparatus:[8]

The mirrored cube (30×30×30 cm) was constructed of 5 pieces of mirrored glass. The mirrored surfaces face the interior of the cube. The container box (40×40×30.5 cm) had a white floor and opaque black walls. Placement of the mirrored cube into the center of the container formed a 5 cm corridor completely surrounding the mirrored chamber. A sixth mirror was placed on the container wall positioned so that it faces the single open side of the mirrored chamber. Luminance in the corridor surrounding the mirrored chamber was 200 lux, whereas within the minor compartment luminance was 100 lux.

Mice were exposed to the chamber of mirrors & evaluated only once to avoid habituation problems. Mice were placed at a single, fixed starting point at the same corner of the corridor and allowed free movement around the corridor and into the chamber of mirrors. During 5-min sessions, the transfer latency, number of entries and time spent in mirrored chamber by mice was observed and recorded. The criterion for entry into the chamber was all four feet being placed on the floor panel of the mirrored chamber.

Light-Dark Model:[8]

The apparatus used was open top wooden box. The box was divided by a barrier possessing a doorway (7.5 x 5 cm), which mice could cross in two chambers of measures (20 x 30 x 35 cm) painted black and illuminated with dimmed red light and a bright chamber (30 x 30 x 35 cm) painted white and illuminated with 100-W white light source. Mice was placed individually in the centre of the light zone and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the light and dark arena. The suppression of exploratory activity in the light compt. caused by bright illumination is antagonised by anxiolytic agents, and anxiogenic agents exacerbate the behavioural suppression. The simplicity of the model provides rapid means for the assessment of the action of agents capable of modifying anxiety.

Statistics:

The results were expressed as Mean ± SEM. For quantitative data, statistical analysis was performed by one way ANOVA by Dunnett test. $P<0.01$ and $P<0.05$ was considered as significant level.

RESULTS:

Elevated plus maze:

Oral administration of 100, 300 & 500mg/kg of *Camellia sinensis* extract produced a significant (P<0.01, ANOVA followed by Dunnett's test) increase in time spent and total arm entries in the open arms of the maze, as compared to control, suggesting an anxiolytic effect of combined extract. Diazepam also significantly increased the amount of time spent and total arm entries in open arm compared to vehicle treated group. (Table No.1)

Table No. 1: Anti-anxiety activity of *Camellia sinensis* extract (CSE) using Elevated Plus Maze

Treatment	Dose (mg/kg)	No. of entries		Avg. Time spent in open arm (Sec)
		Open arm	Closed arm	
Control	-	2.66±0.3333	7±1.528	10.66±2.186
CSE	100	10±0.5774**	6±0.5774	29±0.5774**
CSE	300	14.33±0.3333**	3.33±0.6667*	32±1.155**
CSE	500	17.33±1.453**	1.33±0.3333**	35.33±0.3333**
Diazepam	2	13±0.5774**	4.33±0.3333	32.66±0.3333**

*(Values are expressed as mean±SEM, n=6,*p<0.05, **p<0.01 compared with vehicle control One way ANOVA followed by Dunnet's test)*

Mirrored chamber apparatus:

Oral administration of 100, 300 & 500mg/kg of *Camellia sinensis* extract produced a significant (P<0.01) increase in time spent and number of entries in the mirrored chamber as compared to vehicle treated control mice. Similarly there was a significant (p<0.01) decrease in transfer latency in the mirror chamber. (Table No.2) **Table No. 2: Anti-anxiety activity of *Camellia sinensis* extract (CSE) using Mirror chamber apparatus**

Treatment	Dose (mg/kg)	Transfer Latency (Sec.)	No. of entries	Time spent (Sec.)
Control	-	1119.33±4.055	10±0.5774	416±4.726
CSE	100	689.66±2.906**	15±1.000	820±8.386**
CSE	300	575.66±5.812**	18.33±2.186*	1150±9.539**
CSE	500	425±8.660**	21.33±2.333**	1239.66±5.783**
Diazepam	2	701.33±7.796**	17±0.5774*	859.33±7.513**

*(Values are expressed as mean±SEM, n=6, *p<0.05, **p<0.01 compared with vehicle control One way ANOVA followed by Dunnet's test)*

Light-Dark Model:

Oral administration of 100, 300 & 500mg/kg of *Camellia sinensis* extract produced a significant (P<0.01) increase in time spent and number of entries in the light compartment of light-dark model as compared to vehicle treated control mice. (Table No.3)

Table No. 3: Anti-anxiety activity of *Camellia sinensis* extract (CSE) using Light-Dark Model

Treatment	Dose(mg/kg)	No. of entries in light compartment	Time spent in light compartment (sec)
Control	-	10±1.000	23.66±2.028
CSE	100	13±1.528	97.66±1.453**
CSE	300	15.66±0.8819*	103.33±1.856**
CSE	500	18.66±2.028**	108±2.309**
Diazepam	2	15.66±0.8819*	101±1.528**

*(Values are expressed as mean±SEM, n=6, *p<0.05, **p<0.01 compared with vehicle control One way ANOVA followed by Dunnet's test)*

DISCUSSION:

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic agents are expected to increase the motor activity, which is measured by time spent by the animal in the open arms.

In the present study, we examined the effect of extract of *Camellia sinensis* on anxiety by using elevated plus maze. *Camellia sinensis* extract showed anxiolytic activity in dose-related manner. Extract increase the time spent in open arm and decrease the time spent in close arm indicating anxiolytic activity. The alteration in the time spent in open arm is considered more sensitive to the drug effect than the number of entries. Still, there is also increase in number of entries in open arm.

When a mouse approaches the mirrored chamber, it initially doesn't touch the surface but retreats to the corridor and circles the entire corridor. Then it exhibits a series of partial entries in succession-one foot, two feet, three feet onto the mirrored surface. The typical latency to enter into the chamber of mirrors in a 30-min session is 1000-1200 sec. therefore, the mirrored chamber method is simple to enjoy, nonpunishing, rapid and quantitative and possesses pharmacological attributes which distinguish its response to anxiolytics from other assays of exploratory behaviour. Our studies suggest that the mirrored chamber, which measures a specific pharmacological attribute to anxiety, is as sensitive as the elevated plus-maze for the evaluation of anxiolytics.

Green tea extract showed anti-anxiety activity as evidenced by dose dependent significant increase in number of entries and total time spent in mirrored chamber and also by decreased transfer latency.

It has been concluded that measurement of the time spent in the light zone, but not the number of transfers was the most reproducible and useful parameter for assessing anxiolytic activity. These data seem to be in good agreement with our results. The present data showed that CSE extract increases the time spent in the light zone, suggesting again these extracts possesses anxiolytic properties. The effect of the extract was comparable with diazepam used as a reference drug in the study.

These observations clearly indicate that *Camellia sinensis* extract exerts an anxiolytic activity.

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