

Full Length Research Paper

Anxiolytic and anticonvulsant activity of alcoholic extract of heart wood of *Cedrus deodara roxb.* in rodents

D. Dhayabaran¹, E. Jeyaseeli Florance¹, K. Nandakumar² and A. Puratchikody^{3*}

¹Farooqia College of Pharmacy, Mysore-570 021, India.

²Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal-576 104, India.

³Department of Pharmaceutical Technology, Anna University, Tiruchirappalli-620 024, India.

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The objective of the present study was to evaluate the anxiolytic and anticonvulsant activity of the alcoholic extract of heart wood of *Cedrus deodara* (ALCD). 50, 100 and 200 mg/kg of ALCD were tested for its anxiolytic and anticonvulsant activity. Anxiolytic activity was tested by exposing mice to unfamiliar aversion in different methods like elevated plus maze model, light-dark model and actophotometer. The results suggest that ALCD reduced the aversion fear and produced anxiolytic activity in a dose dependent manner. Pentylentetrazole (PTZ) induced convulsions and maximal electro shock (MES) induced convulsions models in mice were used for assessment of its anticonvulsant activity. 100 and 200 mg/kg of ALCD increased the onset of clonus and tonic seizures in PTZ induced convulsions model and decreased the duration of tonic extensor phase in MES induced convulsions model and also increased the percentage protection in PTZ and MES induced convulsions. Effect of ALCD (30 and 100 mg/kg) on gamma aminobutyric acid (GABA) levels of brain also studied. Estimation of GABA in rat brain after administration of ALCD showed significant modulation of GABA levels. In conclusion these observations suggest that 100 and 200 mg/kg doses of ALCD exhibit anxiolytic and anticonvulsant activity.

Key words: *Cedrus deodara*, anxiolytic, anticonvulsant, gamma aminobutyric acid estimation.

INTRODUCTION

Anxiety and depression are extremely dramatic and debilitating multifaceted disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of depression and anxiety.

Currently the most widely prescribed medications for anxiety disorders are benzodiazepines. But the clinical applications of benzodiazepines as anxiolytics are limited by their unwanted side effects. Therefore the development of new pharmacological agents from plant sources is well justified (Emamghoreishi et al., 2005).

The use of herbal medications by physicians in Europe and Asia is becoming more common and researchers are exploring the traditional remedies to find a suitable cure for these mind affecting diseases (Rabbani et al., 2004). *Cedrus deodara* belonging to the family Pinaceae is a graceful, ornamental evergreen tree growing extensively on the slopes of Himalayas. The genus is comprised of trees which are sometimes cultivated either for their usefulness to traditional cultures or for ornamental purposes. Traditionally the heart wood of *C. deodara* was used to enhance cerebral function, balance the mind, body connection, nervous system and strengthen the brain. It was reported to possess Central Nervous System (CNS) depressant and neuroleptic activity (Nadkarni and Nadkarni, 1996; Kirtikar and Basu, 1991; Agarwal and Rastogi, 1981). So the present study was undertaken to evaluate the anxiolytic and anticonvulsant activity of the alcoholic extract of heart wood of *C. deodara* (ALCD).

*Corresponding author. E-mail: puratchipharma@gmail.com.
Tel: +91 9994372268.

MATERIALS AND METHODS

Drugs and chemicals

Phenytoin (Sun Pharmaceuticals India Ltd., Halol, Gujarat, India). Diazepam (Ranbaxy Laboratories Ltd, HMTD textiles, India), Pentylentetrazole (Sigma-Aldrich, St. Louis, MO63103, USA) were used for the study. All the solvents used for the extraction process were of Laboratory grade and they were purchased from local firms.

Plant extraction

The heart wood of the plant was purchased from the local market in the month of June and authenticated by Dr. K. P. Sreenath, Reader and Taxonomist, Botany Department, Bangalore University and a sample specimen was deposited. The shade dried heart wood of the plant was powdered. The coarse powder was subjected to successive extraction with petroleum ether, alcohol (70%) in Soxhlet apparatus. The percentage yield of petroleum ether and alcoholic extracts were found to be 13.88 and 19.44% respectively.

Phytochemical investigation

The alcoholic extract of heart wood of *C. deodara* (ALCD) was subjected to preliminary qualitative investigations (Khandelwal, 2000).

Experimental animals

Swiss albino mice of either sex (18 - 25 g) and male Wistar albino rats (180 - 200 g) were procured from Bionees, Bangalore and acclimatized at the animal house of Farooqia College of Pharmacy, Mysore. All the animals were maintained under standard conditions, that is room temperature $26 \pm 1^\circ\text{C}$, Relative humidity 45 - 55% and 12:12 h light-dark cycle. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Farooqia College of Pharmacy, Mysore (Approval number IAEC/DD-2/PhD/01-09) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

PHARMACOLOGICAL ACTIVITIES

Acute toxicity studies

Acute oral toxicity of alcoholic extracts of heart wood of *C. deodara* was determined by using female, nulliparous and non pregnant mice weighing 18 - 22 g. The animals were fasted for 3 h prior to the experiment. Up and down procedure organization for Economic Cooperation and Development (OECD) guideline no. 425 was adopted for toxicity studies. Animals were administered with single dose of extract and observed for their mortality during 48 h study period (short term) toxicity. LD_{50} was calculated as per OECD guidelines 425 using AOT 425 software (OECD guidelines no.425, 2001).

Anxiolytic activity

Elevated plus maze model

The apparatus comprises of two open arms (35×5 cm) and two closed arms ($30 \times 5 \times 15$ cm) that extend from a common central

platform (5×5 cm). The floor and walls of the closed arms is wooden and painted black. The entire maze is elevated to height of 50 cm above the floor level. Mice of 18 - 22 g were housed in pair for 10 days prior to testing in the apparatus. During this time the mice were handled by the investigator on alternate days to reduce stress. The animals were divided into five groups of six animals each and received either the control (3% Tween 80, p.o), standard (Diazepam 3 mg/kg, p.o) or ALCD (50, 100 and 200 mg/kg, p.o). 1 h after oral administration of the drug treatment, each mouse was placed in the center of the maze facing one of the enclosed arms. During a five minutes session, the following parameters were noted; Number of entries into open arm and Time spent in the open arm (Hogg, 1996; Rodgers and Johnson, 1998; Pellow and File, 1986). The procedure was conducted preferably in a sound attenuated room.

Light-dark model

Natural aversion of animal for brightly lit places was evaluated in light-dark transition model. Light dark box is a rectangular box of $46 \times 27 \times 30$ cm (l x b x h), which is divided into 2 compartments. 1/3rd is for the dark compartment and 2/3rd is served as light compartment. The animals were divided into five groups of six animals each and received either the control (3% Tween 80, p.o), standard (Diazepam 5 mg/kg, p.o) or ALCD (50, 100 and 200 mg/kg, p.o). 1 h after the drug treatment, the mice were placed individually in the illuminated part of the cage. Number of entries into dark box, Time spent in light box, Total locomotion and Defecation were recorded during the test session of 5 min (Crawley and Goodwin., 1980).

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer. Mice were grouped and treated with drugs as described for elevated plus maze model. Each animal was placed individually and the basal activity score of all the animals were recorded after 30, 60 and 120 min of drug treatment (Alagarswamy et al., 2006).

Anticonvulsant activity

Pentylentetrazole induced convulsion: Male swiss albino mice weighing between 18 - 25 g were divided into five groups each comprising of six animals and received treatments similar to that described for light-dark model. One hour after Vehicle/Standard/Extract treatment, Pentylentetrazole (PTZ) 80 mg/kg was administered intraperitoneally to all the animals. Each animal was placed individually and Latency (onset of clonus), onset of tonic convulsions and Percentage protection were recorded initially for 30 min and at the end of 24 h (Khosla and Pandhi, 2001).

Maximal electroshock induced convulsion

Effect of ALCD was tested against electrically induced convulsions (Swinyard et al., 1952). The mice were placed in a rectangular plastic cage with an open top, permitting full view of animal's motor responses to seizure in the pilot study of various phases of convulsions. A 60A current was delivered transauricularly for 0.2 sec to mice through small alligator clips attached to each pinna by electroconvulsimeter. This current intensity will elicit complete tonic extension of the hind limbs in control mice (Vogel and Vogel.,

2000). Tonic flexion, Tonic extension and percentage protection were recorded, 30 min after the oral administration of the Vehicle (3% Tween 80, p.o) /Standard (Phenytoin 25 mg/kg, p.o)/ALCD (50, 100 and 200 mg/kg, p.o).

Estimation of GABA levels in rat brain

The Effect of ALCD on GABA levels in brain was studied, based on that described previously by other workers (Suher et al., 2000). After seven days treatment with vehicle (3% Tween 80, p.o) or standard (Diazepam 2 mg/kg, p.o) or extract (30 and 100 mg/kg, p.o), animals were killed by euthanasia and the body was exposed to a microwave irradiation for 4 sec. The brain was rapidly removed and the cerebellum, the tissue other than cerebellum was dissected on an ice-cold petri dish. The tissues were weighed and placed in pre-cooled 100 ml plastic tubes. Ice-cooled 0.1 M perchloric acid (10 ml) containing valine at a concentration of 15 µg/ml (internal standard) was added to the tissue. The tissues were homogenized for one minute during which the tube was embedded in an ice bath and then centrifuged at 5000 rpm for 10 min at 4°C. The supernatants were stored at -20°C until assayed. Dansylation reaction was induced by adding 100 µl of each supernatant of the samples or the standards to a micro-tube containing 100 µl of 0.1M potassium carbonate solution. These solutions were mixed using vortex and then centrifuged using microcentrifuge at 10000 rpm for 10 min. 100 µl of each supernatant was transferred into a pyrex tube containing 100 µl of 0.1 M sodium hydrogen carbonate solution, to which 400 µl of working dansyl chloride solution (1.25 mg/ml anhydrous acetone) was added. The tubes were shaken for 30 sec using vortex and then incubated at 90°C in benchtop oven for 30 min. The tubes were not capped during the incubation to allow most of the solvent to be evaporated.

This did not appear to adversely affect the progress of the dansylation reaction and served to concentrate the samples. After getting the tubes out of the oven, they were allowed to cool down to room temperature and the dansylated derivatives were transferred to 1.5 ml micro tubes and stored at -20°C until assayed. C8 reversed-phase High-Performance Liquid Chromatography (HPLC) columns (5 µm, 250 x 3.2 mm) were used to resolve and quantify the samples. The HPLC mobile phase consisted of deionized helium degassed water-acetonitrile (HPLC grade) mixture (65:35 v/v) containing 0.15% v/v phosphoric acid. The flow rate was kept at 0.5 ml/min. The detector excitation was at 333 nm and emission at 532 nm. 25 µl of the dansyl derivative of the Gamma Aminobutyric Acid (GABA) samples were transferred to HPLC micro-sample vials and injected into the column. Retention time of GABA and internal standard were determined. The peak ratios of the samples were calculated with reference to the internal standard. GABA levels were expressed as ng/g of tissue.

Statistical analysis

Values are expressed as mean ± SEM from 6 animals. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Tukey-kramer test. $p < 0.05$ was considered significant. All the analysis was made using the INSTAT statistical software package.

RESULTS

Phytochemical investigation

It was found that the ALCD contains alkaloids,

carbohydrates, proteins, tannins and phenolic compounds.

Acute toxicity studies

Acute toxicity studies were conducted in albino mice according to OECD guidelines no.425 and LD₅₀ of ALCD was computed to be 1098 mg/kg.

Assessment of anxiolytic activity

Elevated plus-maze model

In elevated plus-maze test (EPM), the ALCD at a dose of 100 and 200 mg/kg significantly increased the number of entries (12.67 ± 1.22 and 12.17 ± 1.35) and time spent into the open arm (107.33 ± 2.98 and 114 ± 20.34). The magnitude of the anxiolytic effects of 100 and 200 mg/kg of ALCD was comparable to that of diazepam 3 mg/kg p.o. (Figures 1 and 2).

Light-dark model

In light-dark test (LDT), 100 and 200 mg/kg dose of ALCD has significantly increased the time spent in light box (164.83 ± 15.09 and 171 ± 14.25) and decreased total locomotion (157.5 ± 13.27 and 147.5 ± 15.75), confirming anxiolytic activity of ALCD (Table 1).

Actophotometer

The average Actophotometer reading in the control group was 294.5 ± 16.69 after administration of ALCD 50, 100 and 200 mg/kg after 60 min significantly reduced the locomotors activity (130.16 ± 3.59 , 114.15 ± 7.53 and 72.52 ± 9.95). It may be due to the CNS depressant property of the drug (Table 2).

Assessment of anticonvulsant activity

Pentylenetetrazole induced convulsions: In PTZ induced convulsion model, ALCD 50, 100 and 200 mg/kg doses showed significant alteration in the onset of tonic - clonic seizures compared to control group animals. The protection against the seizures was 16.66, 83.33 and 100% respectively (Table 3). The convulsions were completely abolished by diazepam.

Maximal electro shock induced convulsions

In maximal electro shock (MES) induced convulsions

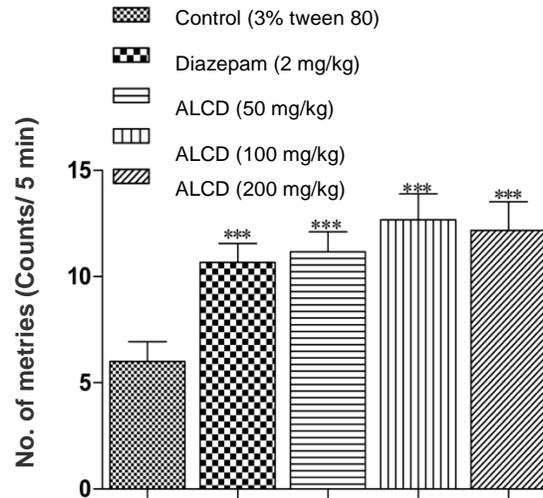


Figure 1. Effect of ALCD on number of entries (open arm) in elevated plus-maze model. Values are expressed as mean \pm SEM, from 6 mice. Significant at *** $p < 0.001$ as compare to control using one-way ANOVA followed by Turkey's post hoc test.

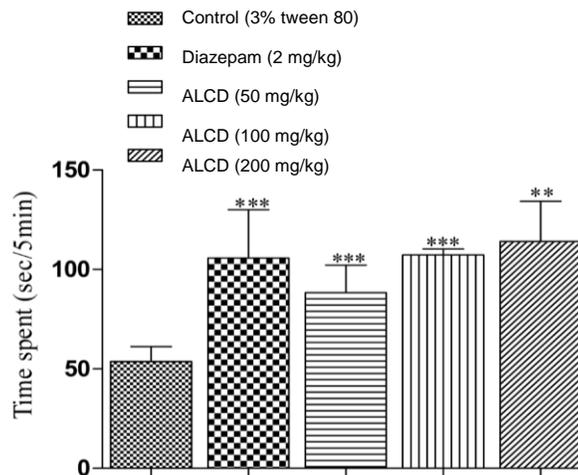


Figure 2. Effect of ALCD on time spent (open arm) in elevated plus-maze model. Values are expressed as mean \pm SEM, from 6 mice. Significant at ** $p < 0.01$ and *** $p < 0.001$ as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

model 50, 100 and 200 mg/kg doses of ALCD exhibited significant anticonvulsant effect, the extract had shown significant decrease in the duration of extensor phase (8.71 ± 0.57 , 7.83 ± 0.63 and 3.34 ± 0.20). It also showed significant increase in percentage protection at 100 and 200 mg/kg doses (Table 4). Phenytoin completely inhibited the duration of tonic extensor phase and protected 100% of animals.

Estimation of GABA levels in rat brain

Pretreatment with ALCD (30 and 100 mg/kg, p.o) for seven days increased the GABA levels in cerebellum (767.42 ± 34.75 and 990.45 ± 43.21 ng/g of tissue) and whole brain other than cerebellum (3083.31 ± 195.15 and 3571.97 ± 204.03 ng/g of tissue). Similar effect was observed with diazepam (2 mg/kg). However, the effect

Table 1. Effect of ALCD on various parameters in light-dark model.

Treatment	No of entries in to dark box (s)	Time spent in light box (s)	Total locomotion (Counts/5 min)	Defecation (Counts/5 min)
Control (3%Tween-80)	9.83 ± 0.79	65.83 ± 5.63	291.67 ± 18.46	5.83 ± 1.72
Diazepam (5 mg/kg)	4.5 ± 0.42 ^{***}	176.83 ± 15.68 ^{***}	131.67 ± 12.69 ^{***}	2.5 ± 0.34 ^{***}
ALCD (50 mg/kg)	5.34 ± 0.66 ^{***}	124.5 ± 18.62 ^{***}	287.5 ± 14.59	4.34 ± 0.61
ALCD (100 mg/kg)	4.16 ± 0.47 ^{***}	164.83 ± 15.09 ^{***}	157.5 ± 13.27 ^{**}	2.83 ± 0.30 ^{**}
ALCD (200 mg/kg)	3.83 ± 0.75 ^{***}	171 ± 14.25 ^{***}	147.5 ± 15.75 ^{**}	2.33 ± 0.21 ^{***}

(Observation period: 5 min for all parameters) Values are expressed as mean ± SEM, from 6 mice. Significant at ^{**}P<0.01 and ^{***}P<0.001 as compare to control using one-way ANOVA followed by Tukey-Kramer's post hoc test.

Table 2. Effect of ALCD on locomotors activity (Actophotometer) in mice at different time intervals (min).

Treatment	Photocell counts		
	30 min	60 min	120 min
Control (3% Tween-80)	294.5 ± 16.69	281.24 ± 11.43	289.56 ± 6.23
Diazepam (3 mg/kg)	80.66 ± 15.36 ^{***}	60.8 ± 9.21 ^{***}	64.33 ± 4.29 ^{***}
ALCD (50 mg/kg)	149.16 ± 12.66 ^{***}	130.16 ± 3.59 ^{***}	151.5 ± 6.42 ^{***}
ALCD (100 mg/kg)	127.14 ± 18.84 ^{***}	114.15 ± 7.53 ^{***}	109 ± 6.88 ^{***}
ALCD (200 mg/kg)	103.33 ± 20.52 ^{***}	72.52 ± 9.95 ^{***}	73.66 ± 8.08 ^{***}

Observation period: 10 min for all parameters. Values are expressed as mean ± SEM, from 6 mice. Significant at ^{***}P<0.001 as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

Table 3. Effect of ALCD on pentylenetetrazole induced convulsions.

Treatment	Latency (Onset of clonus) (S/30 min)	Onset of tonic (S/30 min)	% Protection (24 h)
Control (3%tween80)	49.33 ± 5.32	368.33 ± 17.97	0
Diazepam (5 mg/kg)	0	0	100
ALCD (50 mg/kg,po)	175 ± 12.39 ^{**}	412 ± 39.46 ^{**}	16.66
ALCD (100 mg/kg, po)	265 ± 18.29 ^{***}	564 ± 53.15 ^{***}	83.33
ALCD (200 mg/kg, po)	290 ± 14.18 ^{***}	672 ± 69.46 ^{***}	100

Values are expressed as mean ± SEM, from 6 mice. Significant at ^{**}P<0.01 and ^{***}P<0.001 as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

Table 4. Effect of ALCD on Maximal electro shock induced convulsions.

Treatment	Duration of tonic flexion (s)	Duration of tonic extensor (s)	% protection (24 h)
Control (3% Tween 80)	0	14.17 ± 0.87	16.66
Phenytoin (25 mg/kg)	5.83 ± 0.98	0	100
ALCD (50 mg/kg, po)	0	8.71 ± 0.57	33.33
ALCD (100 mg/kg, po)	0	7.83 ± 0.63 [*]	83.33
ALCD (200 mg/kg, po)	0	3.34 ± 0.20 [*]	100

Values are expressed as mean ± SEM, from 6 mice. Significant at ^{*}P<0.05 and as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

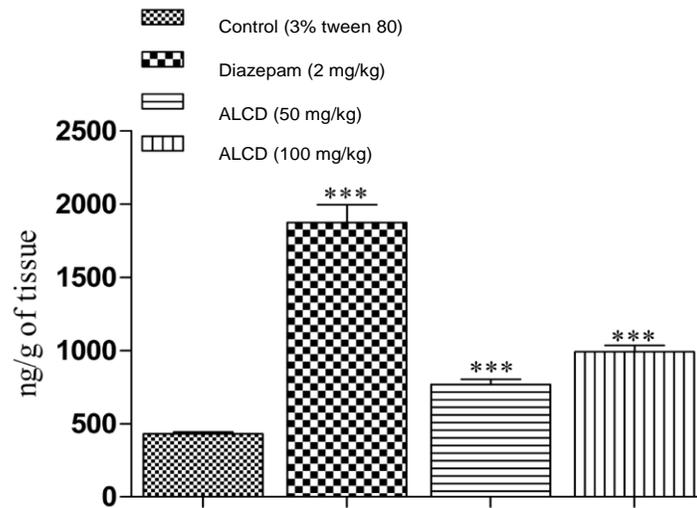


Figure 3. Effect of ALCD on GABA in cerebellum. Values are expressed as mean \pm SEM, from 6 rats. Significant at *** $p < 0.001$ as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

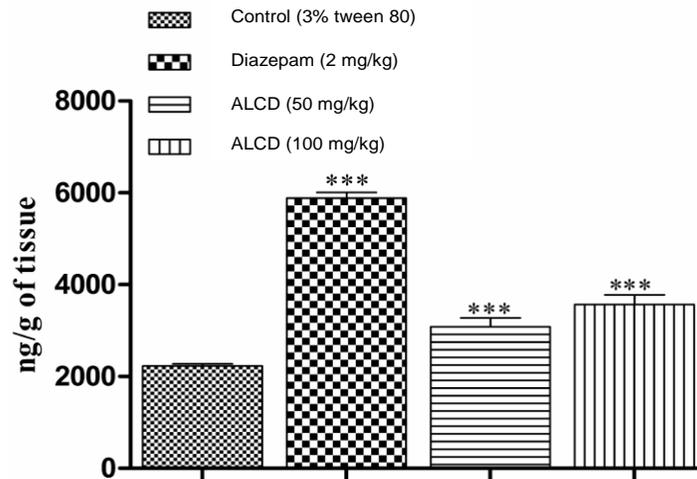


Figure 4. Effect of ALCD on GABA except cerebellum. Values are expressed as mean \pm SEM, from 6 rats. Significant at *** $P < 0.001$ as compare to control using one-way ANOVA followed by Tukey-kramer's post hoc test.

of ALCD was less than diazepam (Figures 3 and 4).

DISCUSSION

Fear and anxiety are defined as the response of a subject to real or particular threats that may impair its homeostasis. This response may include physiological or/and behavioral. Measuring anxiety like behavior in

mice has been mostly undertaken using a few classical animal models of anxiety such as the elevated plus maze and light dark model. All these procedures are based upon the exposure of subject to unfamiliar aversive place (Belzung and Griebel, 2001). Epilepsy is one of the most common serious neurological conditions. Seizure refers to a transient alteration of behaviour due to disordered, synchronous and rhythmic firing of populations of brain

neurons (Noel et al., 2008); the frequency and importance of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age (Marjan et al., 2007). For measuring the anticonvulsant activity in mice has been mostly undertaken using a few classical animal models such as the PTZ induced convulsions and MES induced convulsions (Madhavan et al., 2008; Atif et al., 2005). Studies have proved that the agents which increase the brain GABA content and administration of centrally active GABA mimetic agents have been used as a effective therapeutic approach for treatment of epilepsy, Hence, to see the effect of the extract on GABA levels different parts of the brain, the animals were treated with the extracts and GABA levels were estimated by HPLC method (Suher et al., 2000). The heart wood extracts of *C. deodara* which have been not studied so far for its anxiolytic and anticonvulsant activity. In present study the heart wood extracts of *C. deodara* was studied for anxiolytic and anticonvulsant activity by three experimental models namely elevated plus maze test, light dark model, locomotor activity by actophotometer and anticonvulsant activity was studied by using pentylene tetrazole induced convulsions and MES induced convulsions and pretreatment with ALCD followed by estimation of GABA in rat brain tissues was performed to study the effect of ALCD on GABA levels of brain.

The elevated plus maze is currently one of the most widely used models of animal anxiety (Hogg, 1996; Rodgers and Johnson, 1998; Pellow and File, 1986), the test is principally based on the exposure of animal to an elevated maze array evokes an approach-avoidance conflict that is considerably stronger than that evoked by exposure to an open maze array. The animals being exposed to the new environment tend to avoid open entries and prefer to stay in closed arm due to fear. In our study the ALCD at 50, 100 and 200 mg/kg doses has significantly increased the time spent and number of entries into the open arm indicating the test drugs could reduce the fear and anxiety in the mice. In light dark model, ALCD (50, 100 and 200 mg/kg) has increased the time spent and number of entries into the light compartment. Anxiolytics should reduce the natural aversion to light, the essential feature of this model is that anxiolytic drugs increase the number of crossings and/or the time spent in the light compartment. These results suggests that extract administration could reduce the aversion fear and produce anxiolytic activity. In pentylene tetrazole induced convulsions model (Khosla and Pandhi, 2001) the ALCD (100 and 200 mg/kg) has significantly increased the onset of clonus, onset of tonus and percentage protection when compare to control group and in MES induced convulsions model (Vogel and Vogel, 2000) ALCD (100 and 200 mg/kg) has significantly decreased the duration of tonic extensor and increased the percentage protection when compare to the control group

group. GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders. Many of the traditional agents used to treat psychiatric disorders are known to act, at least in part, by enhancing GABA activity, while some of the newer agents may exert their therapeutic effects exclusively through GABAergic actions. In our present study, seven days treatment with ALCD (30 and 100 mg/kg) and further GABA estimation in brain showed significant enhancement of GABA levels in cerebellum and whole brain other than cerebellum compared to control group.

Conclusion

These findings suggest that the alcoholic extract of heartwood of *C. deodara* possess significant anxiolytic and anticonvulsant activity through modulation of GABA levels in brain. This provides a rationale for its use in traditional medicine for the management of epilepsy and anxiety. Further work is going to isolate the active principle and elucidate the actual mechanism involved in the anticonvulsant and anxiolytic activity of the heart wood of *C. deodara*.

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