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EVALUATION OF BEHAVIORAL EFFECTS OF *CUPRESSUS SIMPERVIRENS* L. ESSENTIAL OIL IN RODENT

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ABSTRACT

The objective of this work was to study the effect of essential oil of Cypress tree on central nervous system by using various neurobehavioural tests. The neurobehavioral effect of the essential oil (CEO) was studied by monitoring its effect on anxiety, locomotion and nociception. In the elevated plus maze apparatus, the number of entries in the open arm of EPM was 6.0 ± 1.2247 at 200 mg/kg CEO and 6.4 ± 0.8944 at 400 mg/kg dose. In the locomotion test using actophotometer, mice treated with CEO were also able to reduce the number of crossings (score) to 294.4 ± 2.4083 at 200 mg/kg dose and 253.6 ± 3.4351 at 400 mg/kg dose. The percent reduction in locomotion was found to be 16.22 and 28.11 % respectively at the low and high dose of CEO suggesting a significant reduction in comparison to the basal score. In order to measure the anti-nociceptive action mediated through the CNS, eddy plate was used. The latency to response was a measure analgesia induced by the drug. Mice treated with CEO were able to enhance the latency period significantly at both the doses. The latency period after 90 min was 5.50 ± 0.0733 sec and 6.48 ± 0.0961 sec for 200 mg/kg and 400 mg/kg respectively while that after 120 min was 5.65 ± 0.0327 and 6.92 ± 0.0686 respectively. This suggest a dose dependent anti-nociceptive action of CEO. The results led to the conclusion that Cypress tree essential oil possess the ability to influence to working of the central nervous system.

Keywords: Cypress, neurobehavior, locomotion, analgesia, anxiety

INTRODUCTION

Cupressus sempervirens L. is an evergreen tree native to scattered localities in Northern Hemisphere, Himalayas, southern China and Africa [1]. Phytochemical evaluation of the tree has revealed the presence of polyphenols, flavonoids and flavone glycosides. Bioflavonoid including amentoflavone, cupressuflavone, 4''-mono-Omethyl amentoflavone, hinokiflavone, isocryptomerin and podocarpusflavone A has also been reported from this plant [2]. Various parts of the plant have been reported to possess antiseptic, aromatherapeutic, astringent, balsamic and anti-inflammatory activities, antispasmodic, astringent, antiseptic, deodorant, and diuretic effects [3-8]. The essential oil of the tree is widely utilized in cosmetic industry and pharmaceutical industry (Ayurvedic) for its skin toning and astringent action, relaxation of stiff muscle and calming of mind.

The presence of terpenes and sesquiterpenes in the essential oil of Cypress tree (CEO) might be the driving factor for its calming benefits. The neurobehavioral screening of drugs is done in three domains including general examination (screen 1), motor function, nociception, anxiety, memory (screen 2) and screening for neuropsychiatric disorders (Screen 3) [9]. To our best

knowledge no scientific study exploring the neurobehavioral effects of CEO has been conducted yet. Hence in the present work the objective was to study the neurobehavioral effect of CEO in rodent using screen 2 tests like elevated plus maze test, learning test by Actophotometer and nociception by Eddy's hot plate method.

MATERIAL AND METHODS

Cypress tree oil was purchased from Veda oils, New Delhi. Other material required for the study were purchased from Central Drug House, New Delhi. Morphine and Diazepam formulation were purchased from local pharmacy. The protocol was approved by the Institutional Animal Ethical Committee.

Physicochemical evaluation of oil

Boiling Point determination

The boiling point of lavender oil was determined by placing the oil in a fusion tube and tying it to a thermometer. A capillary tube was dipped in the oil and the thermometer was dipped in Thiele's tube containing liquid paraffin. Heat was applied to the tube and the temperature at which boiling begins (indicated by bubble formation from side of the capillary tube) was recorded as the boiling point of the oil [10].

Refractive Index

The refractive index of the lavender oil was determined by using Abbe's refractometer. The surface of the prism of refractometer was cleaned with acetone and a drop of oil was added on the lower prism. The lower prism was locked with the upper prism to form a film of the oil. Light was allowed to travel through the prisms and the change in path of the light was measured from the dial of the instrument [11].

Pharmacological Evaluation

Animals

Swiss albino mice (18-22 g) of either sex were used for the study. The animals were housed in polypropylene inclusions using paddy husk for bedding and kept in the animal house at $24 \pm 2^\circ\text{C}$ temperature and 30 – 70 % relative humidity and a 12:12 light: day cycle. All the

animals were freely permitted to water and fed with standard commercial pelleted rat diet. All the investigational procedures and protocols used in this study stood reviewed by the Institutional Animal Ethics Committee.

The test sample was prepared by suspending the oil within 0.5% aqueous solution of methylcellulose containing 0.1% Tween 80. Two doses of oil were administered (200 mg/kg and 400 mg/kg body weight) as oral bolus to the animal.

Anxiolytic activity [12]

The anxiolytic activity of the oil was carried out using elevated plus maze test using diazepam as the reference control. Animal were grouped in the following groups for study (Table 1).

Table 1: Grouping of animals for anxiolytic study of oil

Group	Treatment	No. of Animals
I	0.1% Carboxy Methyl Cellulose (CMC) solution (10ml/kg)	5
II	Diazepam, 1 mg/kg, i.p.	5
III	Cypress tree essential oil (CEO) 200 mg/kg, p.o	5
IV	Cypress tree essential oil (CEO), 400 mg/kg, p.o	5

The test drugs given 30 minutes before commencement of study. The animals were kept in the middle of elevated plus maze one by one and observed the number of open and closed arm entries. The time period spent in open and closed arm by the mice was recorded.

Learning behavior and locomotion [13]

An actophotometer is used to determine the neurobehavioral activities and motor coordination in mice. Animal were grouped in the following groups for study (Table 2).

Table 2: Grouping of animals for learning behaviour of oil

Group	Treatment	No. of animals
I	0.1% Carboxy Methyl Cellulose (CMC) solution (10ml/kg)	5
II	Diazepam, 1 mg/kg, i.p.	5
III	Cypress tree essential oil (CEO) 200 mg/kg, p.o	5
IV	Cypress tree essential oil (CEO), 400 mg/kg, p.o	5

Each animal was placed in the actophotometer to record the basal score for a duration of 5 min. Each time the animal blocks the path of light from the photocell in the actophotometer, the display records a reading. After 30 min of drug administration, each animal was again kept in the actophotometer for 5 min and the number of movements made by the animals was recorded by directly reading the value

from the display. After taking reading from each animal activity in the instrument, the surface was cleaned with ethanol 5% solution.

Antinociceptive activity [14, 15]

The antinociceptive activity of the essential oil was evaluated using Eddy's hot plate method. Animal were grouped in the following groups for study (Table 3).

Table 3: Grouping of animals for antinociceptive study of oil

Group	Treatment	No. of animals
I	0.1% Carboxy Methyl Cellulose (CMC) solution (10ml/kg)	5
II	Morphine, 5 mg/kg, i.p.	5
III	Cypress tree essential oil (CEO) 200 mg/kg, p.o	5
IV	Cypress tree essential oil (CEO), 400 mg/kg, p.o	5

Mice were placed on the hot plate, which consists of electrically heated surface. Temperature of the hot plate was maintained at 55°C. Responses such as jumping, withdrawal of the paws and licking of the paws were observed. The time period (latency period) when animals were placed and until responses occur was recorded by the stopwatch. The latency period was recorded after 0, 30, 60, 90 and 120 min of administration of the treatment. The values

were compared with the standard drug morphine and control CMC.

RESULTS AND DISCUSSION

Physical characterization

The results of organoleptic characterization and boiling point are presented in Table 4.

A similar organoleptic characteristic of cypress oil are reported in the literature. The solubility was determined and was found in consonance with the reports in the literature [16].

Pharmacological Evaluation

The LD₅₀ of Cypress essential oil components have been previously reported and the data obtained by these study has been utilized to select the dose of oil for the present investigation. The oral LD₅₀ of alpha pinene was 3700 mg/kg; terpinolene was 4390 mg/kg; and camphene was more than 5000 mg/kg in rats¹⁶. Considering the high LD₅₀ value of its components a dose of 200 mg/kg and 400 mg/kg was selected for studying the effect of the essential oil on neurobehavioural parameters.

Anxiolytic Activity

The effect of CEO on mice in elevated plus maze was studied and the results are presented in **Table 5**.

The number of entries in open and closed arm in the control group was 2.8 ± 0.8366 and 5.6 ± 0.8944 respectively. Animal treated with CEO exhibited an increased number of entries in the open arm and decreased the entry of mice in the closed arm suggesting a decreased anxiety in the mice. The number of entries in the open arm of EPM was 6.0 ± 1.2247 at 200 mg/kg CEO and 6.4 ± 0.8944 at 400 mg/kg dose. This suggested that the effect on reducing the anxiety by the essential oil was not dependent on dose of the extract. On the other hand the number of entries in the closed

arm of EPM was 2.4 ± 0.5473 at 200 mg/kg CEO and 1.6 ± 1.1401 at 400 mg/kg dose.

The results revealed that the number of entries in the open arm was significantly improved while the reduction in number of entries in the closed arm was not significant in comparison to diazepam. The elevated plus-maze is a validated predictive test for anxiety-like behavior of rodents in which the animal prefers to stay in the closed arms rather than open arm. The CEO was able to significantly cause CNS depressant effect in mice as exhibited by the positive response in elevated plus-maze.

Learning and Locomotion

The effect of CEO on locomotor activity was observed using Actophotometer and the results are given in **Table 6**. The reference drug Diazepam significantly reduced the number of crossings of light beams to 200.6 ± 5.3665 post 30 min of administration exhibiting a reduction of 43.87%. Mice treated with CEO were also able to reduce the number of crossings (score) to 294.4 ± 2.4083 at 200 mg/kg dose and 253.6 ± 3.4351 at 400 mg/kg dose. The percent reduction in locomotion was found to be 16.22 and 28.11 % respectively at the low and high dose of CEO suggesting a significant reduction in comparison to the basal score.

The reduction in locomotion by CEO exhibits the potential of the essential oil to cause a significant depressant action in the central nervous system. Actophotometer is used to study the effect of drug on CNS observed by the number of movements that the test animal makes during a period of 5 min of the study. The activity is a measure of the level of excitability of the CNS; decreased activity results from depression of the central nervous system. The percent reduction in locomotion was calculated from the basal score and is comparatively represented in **Figure 1**.

Antinociceptive activity

Response against thermal inducer of nociception is widely accepted as a method to study the analgesic (antinociceptive) activity of drugs. The effect of CEO on thermal induced pain was observed using Eddy's hot plate (analgesiometer) and the results are

given in **Table 7**. The latency to response was a measure analgesia induced by the drug. The reference drug Morphine significantly increased the latency period for response to heat exhibiting a latency of 9.89 ± 0.0684 sec as compared to 1.25 ± 0.0288 sec in the control group after 120 min of administration. Mice treated with CEO were also able to enhance the latency period significantly at both the doses. It was found that maximum analgesic action of the Cypress essential oil was found after 90 min with not much change in the next 30 min. The latency period after 90 min was 5.50 ± 0.0733 sec and 6.48 ± 0.0961 sec for 200 mg/kg and 400 mg/kg respectively while that after 120 min was 5.65 ± 0.0327 and 6.92 ± 0.0686 respectively. This suggest a dose dependent anti-nociceptive action of CEO.

Table 4: Organoleptic properties of cypress oil

Test	Specification	Observation
Color	Colorless to pale yellow	Pale Yellow
Odor	Characteristic	Characteristic
Refractive Index	1.468-1.478	1.465
Boiling Point	176°C	171-174°C
Specific gravity	0.892-0.950	0.899

Table 5: Effect of CEO on mice in EPM

Treatment	Dose	Number of open arm entries	Number of close arm entries	Total arm Entries
Control	10 ml/kg, p.o.	2.8 ± 0.8366	5.6 ± 0.8944	8.4 ± 1.6733
Diazepam	1 mg/kg, i.p.	11.2 ± 1.0954	0.8 ± 1.0954	12.0 ± 1.7320
CEO	200 mg/kg, p.o.	6.0 ± 1.2247	2.4 ± 0.5473	8.4 ± 1.6733
	400 mg/kg, p.o.	6.4 ± 0.8944	1.6 ± 1.1401	8.0 ± 1.2247

Values are represented as mean \pm SD; n = 5

Table 6 : Effect of CEO on basal score in actophotometer

Treatment	Dose (mg/kg)	Basal Score	Score after 30 min
Control	10 ml/kg, p.o.	355.6 ± 3.4351	348.6 ± 2.7018
Diazepam	1 mg/kg, i.p.	357.4 ± 1.6733	200.6 ± 5.3665
CEO	200 mg/kg, p.o.	351.4 ± 3.0495	294.4 ± 2.4083
	400 mg/kg, p.o.	352.8 ± 2.8635	253.6 ± 3.4351

Values are represented as mean ± SD; n = 5

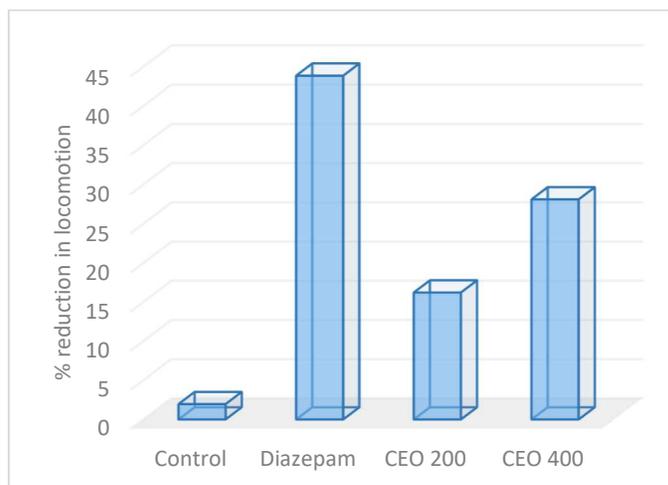


Figure 1: Percent reduction in locomotion in various groups over 30 min

Table 7: Antinociceptive action of CEO in Eddy's hot plate test

Treatment	Dose (mg/kg)	Latency Period (sec)				
		0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg, p.o.	0.98 ± 0.0707	1.12 ± 0.0563	1.19 ± 0.0286	1.23 ± 0.0449	1.25 ± 0.0288
		1.14 ± 0.0922	5.40 ± 0.1454	6.93 ± 0.0350	7.96 ± 0.0669	9.89 ± 0.0684
CEO	200 mg/kg, p.o.	1.02 ± 0.0782	2.4 ± 0.0915	3.67 ± 0.1452	5.50 ± 0.0733	5.65 ± 0.0327
	400 mg/kg, p.o.	1.01 ± 0.0377	3.41 ± 0.0583	4.49 ± 0.0719	6.48 ± 0.0961	6.92 ± 0.0686

Values are represented as mean ± SD

It was found from the results that the essential oil was not able to induce analgesia immediately after administration and the analgesic effect began only post 30 min of administration of the extract whereas morphine owing to intraperitoneal administration was able to exhibit analgesia immediately on administration.

CONCLUSION

The fact that herbal medicines have less negative effects and reactions on the body makes them extremely valuable. In this paper, neurobehavioral investigations were conducted on the essential oil extracted from the needle and stem of the cypress tree. The study's findings showed that the extract had the same effects as diazepam in the elevated plus maze test for anxiolytic activity and the

actophotometer study for locomotion, demonstrating its ability to influence the central nervous system. Through CNS-based pathways, the essential oil was also able to cause analgesia in the animals.

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