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Original Research Article

Anti-anxiety activity of *Eucalyptus tereticornis* n-hexane extract in Wistar albino rats

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ABSTRACT

Background: To find out a new agent with a high therapeutic index for the treatment of anxiety, an indigenous medicinal plant *Eucalyptus terteticornis* was screened for its effect on anxiety in experimental animal model.
Methods: Thirty six adult Wistar albino rats of both sexes weighing 175-200g were divided into three groups: Group I: DMSO 10% (0.1ml/200g), Group II: hexane extract of leaves of *Eucalyptus terteticornis* (ETHE) (100mg/kg/body weight), Group III: Diazepam (1mg/kg orally). All test compounds were administered orally for ten days. On tenth day, after one hour of test compounds administration, Wistar rats were taken for elevated plus maze (EPM) and light dark arena (LDA) tests. Statistical comparisons among the groups were performed by One-way analysis of variance (ANOVA) followed by Tukey Krammer test.
Results: The results showed that ETHE treated animals (Group II) significantly ($p < 0.001$) increased the time spent in open arms of EPM and in bright arena of LDA on comparing with normal (Group I).
Conclusions: The anti-anxiety activity of *Eucalyptus terteticornis* can be due to its effect on brain neurotransmitters or due to antioxidant property.

Keywords: Anti-anxiety, *Eucalyptus terteticornis*, Hexane extract, Wistar rats

INTRODUCTION

Anxiety is described as a frame of mind apprehensive about future in association with preparation for possible, upcoming undesirable happenings. Slight anxiety can assist people perform at their best. Anxiety becomes a medical concern, when it becomes more intense, and interferes with day to day activities. The word anxiety covers several different forms of a common psychiatric disorder which is characterized by excessive ponderings, worrying, restlessness, nervousness and fear about future uncertainties based on real or imagined events, which may affect both physical and psychological health of an individual. Several factors can cause anxiety. They include stress, alcohol and substance abuse, drug induced and genetic abnormalities. Generalized anxiety disorder, panic disorders with or without agoraphobia, social phobia, obsessive compulsive disorder, post-traumatic stress disorder are the examples of different types of anxiety disorders. Currently, selective serotonin reuptake

inhibitors (SSRIs) and benzodiazepines are the preferred drugs for the treatment of anxiety disorders. Although many drugs are available in allopathic medicine to treat anxiety disorders, they are known to produce adverse drug reactions at normal therapeutic doses upon chronic use. In traditional medicine, many plants have anxiolytic activity.[1-8]

Eucalyptus tereticornis is a fast-growing tree up to a height of 30 to 45 m and 1 to 2 m in diameter. It belongs to the family of *myrtacea*. It is known as "forest red gum", and is used traditionally for various ailments. *Eucalyptus* species contains numerous phytochemicals such as alcohols, phenols, terpenes and ketones which are playing a key role of their medicinal properties. Recently an in-vitro study, conducted in our laboratory, showed that the n-hexane extract of *Eucalyptus tereticornis* had a potent antioxidant activity.[9-11]

Experimental evidence of *Eucalyptus tereticornis* having anxiolytic property is lacking. Therefore, this study is undertaken to evaluate the effect of this indigenous medicinal on anxiety-like behaviour in rats. Two pharmacologically validated experimental models, elevated plus maze and light and dark box are employed for screening its effect on anxiety.

METHODS

Animals

Adult Wistar albino rats of both sexes weighing 175-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (YU-IAEC 4/2015), Yenepoya University.

The rats were maintained under standard conditions in the Animal House (CPCSEA approved, Reg. No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were kept in polypropylene cages (U.N. Shah manufacturers, Mumbai) and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water ad libitum. The rats were maintained on a 12:12 hour light-dark cycle.

Drugs

Diazepam (Cipla Ltd.) was obtained from Yenepoya Hospital Pharmacy in Mangalore. It was administered at a dose of (1mg/kg orally).

Instruments

Soxhlet apparatus was used to prepare the plant extract. Elevated plus maze apparatus and Light Dark Arena apparatus for screening anxiolytic activity.

Plant material

The leaves of *Eucalyptus tereticornis* was collected from the local areas of Bellary region of Karnataka State, India during February 2014. They were authenticated by a botanist before preparing the extract. The leaves were shade dried and then ground to a coarse powder.

Preparation of the extract

Eucalyptus tereticornis n-hexane extracts (ETHE)

A weighed quantity (500g) of the coarse powder was taken and extracted with n-hexane in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60ºC. The percentage yield of the extract was 10%. The n-hexane extract was suspended in dimethyl sulfoxide (DMSO). ETHE was administered at a dose of 100 mg/kg/day orally. This dose of the plant extract was decided after doing the acute toxicity study. LD_{50} study showed that ETHE at a dose of 1000mg/kg body weight showed signs of toxicity like aggressive behaviour. So in this study $1/10^{th}$ of 1000mg/kg body weight was selected i.e., 100mg/kg/day.

Experimental design

Animal models form the backbone of preclinical research on the neurobiology of psychiatric disorders, and are employed both as screening tools in the search for novel therapeutic agents and as simulators for studies on underlying mechanisms.[12-14] Thirty six animals were used in this study. The animals were divided into three groups. Each group consisting of 6 males and 6 females (n=12).

Group I: 10% DMSO (0.1ml) orally for 10 days

Group II: ETHE (100 mg/kg/day orally) for 10 days

Group III: Diazepam (1 mg/ kg orally) for 10 days

On 10^{th} day, after an hour of administration of test compounds, the animals were taken for the following tests for screening their anxiolytic activity. The tests are mention below:

Elevated plus maze (EPM)

This test has been widely validated to measure anxiety in rodents. The plus-maze combines three potential anxiogenic factors- novelty, height and opens space. Briefly, the cross-shaped maze consists of four arms that are interconnected by a central platform. Two opposing arms are surrounded by side- and end-walls (closed arms), whereas the remaining two arms are unprotected (open arms). The set-up consists of a maze of two open arms (25cm×5cm), crossed with walls (35cm high) and central platform (5cm×5cm). The maze is suspended 50 cm above the room floor. The animal is placed on the

central platform, facing one of the enclosed arms and observed for 5 minutes. During the 5-min test period, the time spent in open and enclosed arms, was recorded. [5]

Light Dark Arena (LDA)

Light-dark exploration test is one of the few tests specifically designed for use in rats. The original maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment) whereas the remaining 2/3 was open and illuminated (light compartment). The door between the two compartments permits rat to move from one side to another. The rat is released in the light compartment and observed for 5 minutes. During that time the time spent in light and dark compartment, was recorded.[5]

Statistical analysis

Results were expressed as mean±SD. One-way analysis of variance (ANOVA) was carried out and the statistical comparisons among the groups were performed with Tukey Krammer test using Prism statistical package program. $p<0.05$ was considered significant.

RESULTS

Elevated plus maze

ETHE treated animals (Group II) significantly ($p<0.001$) increased the time spend in open arms (Table 1) of EPM on comparing with the normal (Group I). But there was no significant difference between the ETHE treated animals (Group II) and Diazepam treated ones (Group III).

Table 1: Effect of ETHE on the time spends in the arms of elevated plus maze.

Group	Drugs	Time spend in each arm in seconds	
		Open	Closed
I	DMSO	7.01±0.40	272.35±3.21
II	ETHE	56.26±2.90***	150.06±5.53***
III	Diazepam	52.58±1.68***	160.27±7.21***

One Way ANOVA, followed by Tukey Kramer multiple comparison test
Results were expressed as mean ± SD; n= 12.
*** p <0.001 →extremely significant, on comparing groups II and III with group I
DMSO: dimethyl sulfoxide
ETHE: *Eucalyptus tereticornis* n-hexane extract

Light dark arena

ETHE treated animals (Group II) significantly ($p<0.001$) increased the time spend in bright arena (Table 2) of LDA on comparing with the normal (Group I). But there was no significant difference between the ETHE treated animals (Group II) and Diazepam treated ones (Group III).

The above two screening methods proved that *Eucalyptus tereticornis* n-hexane extract has anxiolytic activity.

Table 2: Effect of ETHE on the time spent in the light dark arena.

Group	Drugs	Time spend in each arena in seconds	
		Light	Dark
I	DMSO	30.01±7.332	261.04±4.54
II	ETHE	128.29±4.32***	150.89±7.51***
III	Diazepam	122.53±3.44***	159.73±4.35***

One Way ANOVA, followed by Tukey Kramer multiple comparison test
Results were expressed as mean±SD; n= 12.
*** p <0.001 →extremely significant, on comparing groups II and III with group I
DMSO: dimethyl sulfoxide
ETHE: *Eucalyptus tereticornis* n-hexane extract

DISCUSSION

A very little information is available on the CNS activity of *Eucalyptus tereticornis*. In the present study, n-hexane extract of *Eucalyptus tereticornis* showed significant anxiolytic activity experimental animal models as evidenced by the increase in time spend in open arms and light arena of EPM and LDA.

Anxiety is common in humans. Each individual experiences occasional or situational anxiety symptoms. But in many, it affects day to day activities. Diagnosable anxiety disorders are the most common mental health disorders.[15] These disorders can gravely affect quality of life. The comorbid factors of anxiety disorders are secondary depression or substance abuse. Various diseases like thyroid disease, respiratory disease, gastrointestinal disease, arthritis, migraine headaches, and allergic conditions are associated with anxiety disorder.[16,17]

The exact cause of anxiety ailments is not fully known. Low level of Gamma-amino butyric acid (GABA) in CNS is one of the main causes of anxiety disorders. Apart from GABA, Serotonin (5-HT), Nor-epinephrine (NE) and dopamine also plays a vital role in the patho-physiology of anxiety disorders. Anxiety disorders can be due to reactive oxygen species induced damage to neurotransmitter systems.[5]

The anxiolytic activity of *Eucalyptus tereticornis* can be due to its GABA agonistic activity or by antioxidant property. Its action via modulating other neurotransmitters like 5-HT, NE and dopamine cannot be ruled out.

CONCLUSION

Further studies are on-going to elucidate the exact mechanism by which this plant acts as an anxiolytic

agent. This study is further extended to find out to the levels of neurotransmitters in the brain after the administration of *Eucalyptus tereticornis*. This will give an input about the possible mechanism of action by which this indigenous plant exhibits anxiolytic activity.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethical Committee Clearance (YU-IAEC 4/2015), Yenepoya University

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