ORIGINAL RESEARCH PAPER

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KEY WORDS: Cananga ododrata , Elevated plus maze , anxiolytic,

EVALUATION OF ANXIOLYTIC ACTIVITY OF CANANGA ODORATA LEAVES IN SWISS ALBINO MICE

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ABSTRACT

Cananga Odorata plant is on one of the most commonly used plants in aromatherapy. This study intends to demonstrate anxiolytic action of Ethanolic Extract of Cananga Odorata Leaves (EECOL) in Swiss Albino Mice using Elevated plus maze . Animals were divided into 5 groups with 6 animals (n=6) each. Diazepam was used as standard drug. EECOL was given by oral feeding for test groups at doses of 100mg, 200mg, and 400mg/kg respectively. Animals were placed in elevated maze and allowed to move freely for 300seconds. Entry into the open and closed arms, time spent in open and closed arm were noted.

It was observed that the number of entries and time spent by EECOL treated animals in the open arm increased significantly compared to control group. The study suggests that EECOL has anxiolytic activity on Swiss Albino Mice

INTRODUCTION

Anxiety is a feeling of dread, fear, or apprehension often with no clear justification. Associated with worried thoughts and changes like increased blood pressure, palpitation etc. Anxiety is a normal human emotion that serves an adaptive function from a psychobiological perspective[1]. Globally more than 260 million people are living with anxiety disorders [2]. According to national mental health survey of India 2015-16 Neurosis and stress related disorders affected 3.5% of the population [3]. Current first-line treatments options for anxiety disorders include SSRI SNRI medication, with benzodiazepines best suited for short-term and adjunctive anxiety. TCAs and MAOIs are effective but patient tolerance issues limit their use [4&5]. In addition to their anxiolytic effects, benzodiazepines produce other cns effects like sedation, hypnosis, and muscle relaxant effects. The benzodiazepines also impair cognitive performance and memory, adversely affect motor control, and potentiate the effects of other sedatives including alcohol[6] .This has prompted for the need of newer , more effective drugs of plant origin which have anxiolytic activity with less adverse effects and affordable for low socio-economic patients

Cananga odorata [ylang-ylang], is a fast growing tree found natively in tropical Asia. It is well-known for its fragrant flower and has been introduced to China, India, Africa, and America. Cananga Odorata essential oils are widely utilized in the food industry , perfume industry and as a component of aromatherapy [7]. The medicinal properties exhibited by Cananga Odorata oil is one of the main factors for its popularity in aromatherapy. Studies have shown its antiseptic, anti-inflammatory, mood stabilizing, hypoglycemic effects amongst others. Most notably it has also shown anxiolytic effect as a part of aromatherapy [8]

This study intends to evaluate anxiolytic property of cananga odorata leaves in experimental animals using anxiety model.

MATERIALS & METHODS

Institutional Animal Ethics Committee permission was taken and all the guidelines of CPCSEA were followed throughout the study.

Plant material

Cananga Odorata plant leaves were obtained from AGHP enterprises Chennai.

Preparation of Extract

Ethanol was used as solvent. Ethanolic extract of Cananga Odorata leaves was extracted from Soxhlet apparatus[9] . The extract was dried and sterilized using hot air oven. 80gram of leaves yielded 20g of extract.

Phytochemical analysis

The Ethanolic extract was routinely analysed for alkaloids, carbohydrates, flavonoids, tannin, glycosides, steroids,Saponin and phenol. The phytochemistry of Cananga Odorata is well documented. Cananga Odorata essential oil was shown to contain mainly monoterpene hydrocarbons, oxygen- containing monoterpenes, sesquiterpene hydrocarbons, oxygen- containing sesquiterpenes, benzenoids, acetates, benzoates, and phenols[8]

Experimental animals

Swiss Albino mice were procured from Central Animal House , SNMC Bagalkot. The animals were let to acclimatize to the laboratory atmospheric condition for a week at a temperature of 23±2 degree Celsius, 50-60% humidity with regular 12 hour light cycle.

Drugs & dosage :

Diazepam at the dose of 1mg/kg body weight intraperitonially was used as standard drug{10}.EECOL,the was given at doses of 100,200,400mg/kg body weight{11}. Normal saline was used as control.

Grouping & study design:

Male Swiss Albino Mice weighing 25-30g were selected .Six Swiss Albino Mice were randomly selected and placed in each group{n=6}

TABLE 1: Grouping

Group 1	Control : Normal saline
Group 2	Standard : Diazepam 1mg/kg i.p
Group 3	EECOL 100mg/kg P.o
Group 4	EECOL 200mg/kg P.o
Group 5	EECOL 400mg/kg P.o

Elevated Plus Maze

One of the most commonly used ethological tools used as anxiety models in rodents [12].Elevated plus maze (EPM) consists of two open arms measuring 35x5 cm, crossed with two closed arms of 35x5x20 cm each. The arms are connected at central square of 5x5 cm. The apparatus is elevated at a height of 25 cm above the floor in a dimly illuminated room.[10].

Elevated plus Maze is used based on the following

principles{13} -1}natural aversion to open spaces, 2}conflict between exploration and this aversion.3}elements of neophobia, exploration, and approach/avoidance conflict. The order preference for the mice is closed centre open, indicative of a favouring for relatively secured sections of the maze. This tendency is suppressed by anxiolytic agents.

The elevated plus maze model has a high predictive validity for screening anxiolytic drugs. Anxiolytic drugs increase, and anxiogenic drugs decrease, the number of entries into the open arms and the time spent there. Total entries score is an index of anxiety, and the percentages of entries and time spent in each arm constitute the index of primary anxiety[14]

PROCEDURE

The study was carried out between 09:00 a.m. and 02:00 p.m. in quiet , well illuminated lab . The animals were fasted 12 h prior to the study . 1 h prior to the administration of the drugs, mice were moved into the lab to get acclimatized to the experimental environment[10].After drug administration each mouse was placed at the center of elevated plus maze with its head facing towards open arm. It is allowed to move freely and observed for 5 min[300 seconds] . The entry into open and closed arms and time spent in each arm was video recorded[15]. The entry is considered only when the mice placed all its four limbs on the respective area [16&17]. The number of entry into open arm and closed arm was recorded for and time spent in open arm and closed is recorded.

STATISTICAL ANALYSIS

- Data was analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor. p value < 0.05 was considered as statistically significant .Post-hoc comparisons were made using Least Significant Difference (LSD) test.
- Results & Discussions

[Table 2: results of different groups]

Group	Entries in Open arms	Entried in Closed arms	Time spent in Open arms	Time spent in Closed arms
Control	3.67±1.033	6.67±1.633	41.50±8.191	258.50±8.191
Standard	10.00±.632	8.17±1.835	101.00±2.098	226.00±22.891
Eecol 100mg	3.83±2.229	7.17±1.472	42.83±6.242	250.67±4.590
Eecol 200mg	5.83±2.137	7.50±1.871	52.67±4.274	247.33±4.274
Eecol 400mg	7.83±1.722	10.83±2.137	72.00±3.578	228.00±3.578

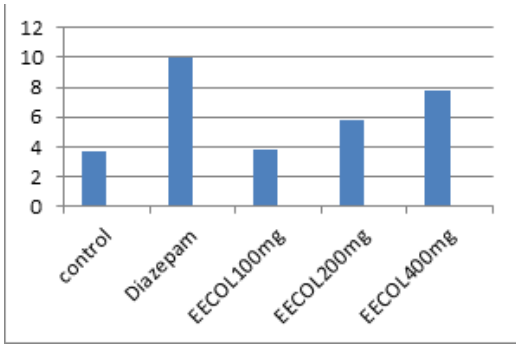
Table 3: Comparison of effect of different groups

		Sum of Squares	df	Mean Square	F	P.
Entries in Open arms	Between Groups	175.533	4	43.883	15.710	.000
	Within Groups	69.833	25	2.793		
Entried in Closed arms	Between Groups	64.533	4	16.133	4.959	.004
	Within Groups	81.333	25	3.253		
Time spent in Open arms	Between Groups	14974.333	4	3743.583	132.25	.000
	Within Groups	707.667	25	28.307		
Time spent in Closed arms	Between Groups	4966.533	4	1241.633	9.652	.000
	Within Groups	3216.167	25	128.647		

Entry into open arms

The number of entry into the open arms increased significantly in Diazepam and EECOL treated groups[200mg, 400mg] compared to control group

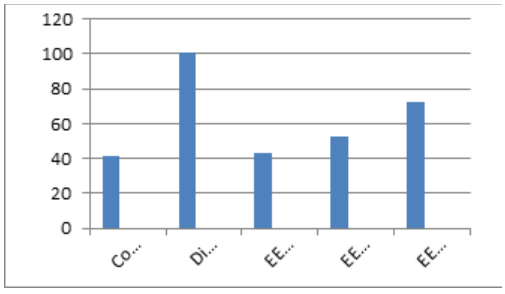
[Graph 1: Graph showing number of entries into open arms]



Time spent in open arms

The time spent in the open arms also increased significantly in Diazepam and EECOL treated groups compared to control

[Graph2: Graph showing time spent in open arms]



CONCLUSION & DISCUSSION

From above analysis it can be concluded that EECOL has anxiolytic activity on Swiss Albino Mice . The anxiolytic activity produced by EECOL at higher doses{200mg and 400mg} is comparable to the standard drug. Many studies have shown the action of Cananga Odorata as having anxiolytic action but studies on exact mode of action are in need.

Essential oils like those of Cananga odorata exhibit different pharmacological actions , like antinociceptive, anxiolytic-like, and anticonvulsant effects. They are widely being used as a complementary therapy for anxiety, insomnia, convulsion, pain, and cognitive deficit symptoms mainly by inhalation and aromatherapy. Recent studies show that essential oils are emerging as a promising source for modulation of the GABAergic system and sodium ion channels{18}. Hence further studies on them can be a promising solution for better treatment options.

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