Original Article

Pharmacological evaluation of anxiolytic property of aqueous root extract of *Cymbopogon citratus* in mice

Abstract

Aim: This study was designed to evaluate the anxiolytic property of aqueous root extract of *Cymbopogon citratus* in mice. **Materials and Methods:** In this study, stress induced hyperthermia (SIH), elevated plus maze (EPM) and open field experimental models were employed. **Results:** In SIH model, the extract caused a significant ($P < 0.01$) reduction in the body temperature (T_2 and ΔT) at doses of 400 mg/kg and 600 mg/kg. Similar reduction in the body temperature was obtained by diazepam 2.5 mg/kg used as standard, while increase in the body temperature (T_2) was observed in the normal saline group. In EPM model, experimental doses of 200, 400 mg/kg of the extract and 2.5 mg/kg of diazepam produced significant ($P < 0.05$) increase in time spent in the open arms when compared to the normal saline group. However, extract dose of 600 mg/kg had no significant ($P > 0.05$) effect. In open field model, 200 mg/kg and 600 mg/kg extract doses significantly ($P < 0.05$) increased locomotion of the mice more than the standard, while rearing and defecation were less in the extract groups. **Conclusion:** In different experimental models used significant anxiolytic effect was observed of the aqueous extract at different dose levels in comparison to reference standard and normal saline group. This clearly justified its folkloric application in the treatment of anxiety disorders.

Key words:
Cymbopogon citratus, diazepam, elevated plus maze and open field model, stress induced hyperthermia

Introduction

The biology of anxiety is considered as an integral part of the human response to threat or danger and it affects about one-eight of the world's population.[1] Anxiety is a generalized response to a potential threat, danger, fear, or even internal or external,[2] and it's marked by a rise in arousal, expectancy, autonomic and neuroendocrine activation and also shows specific patterns in behavior.[3] These changes basically are to enhance coping with an adverse or unexpected situation. Anxiety may also be referred to an elaborate form of fear, and causes an increase in the ability of an individual to adapt and plan for the future.[4] Fear and anxiety are difficult to distinguish. The distinction between the two lies in the concept that the former is a response to an actual threat while latter is anticipatory response to a potential threat.[5,6] The cause of anxiety remains unclear,[7] but report elicits evidence that implicate societal factor, psycho-stimulant drugs (amphetamine, caffeine), genetic and environmental factors.[3] Some anxiety states are essential mechanism for survival and are feature of all mammals.[8]

Barbiturates, alcohol and benzodiazepines have been used in the treatment of anxiety. However, benzodiazepines are now the most commonly used drugs. The clinical use of benzodiazepines (like diazepam) in the treatment of anxiety disorders have been presented with limitations due to their adverse effects which includes psychomotor impairment, potentiating of other central depressant drugs and dependence.[9] This has prompted the search for potent anxiolytics with fewer adverse effects.

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In Nigeria, the use of medicinal plants is well acknowledged and established as a viable profession.[10] *Cymbopogon citratus* commonly known as lemon grass is a tall aromatic perennial plant which grows up to 1.5 m, cultivated by the natives of Asia, parts of South America and in tropical regions of the world. The plant has been used in folkloric medicine in the treatment of various illnesses. In Nigeria, concoction preparation of lemon grass has been used in the treatment of ailments like typhoid, fever, stomach aches,[11] essential oil obtained from lemon grass can help reduce tension and stress.[12] Several scientific investigations have justified diver's folkloric applications of *Cymbopogon citratus*. Study shown that lemon grass can reduced cardiac rate without altering the contractile force in isolated rat heart,[13] report also has it that essential oil of lemon grass produced a marked CNS depression in mice similar to chlorpromazine effect, and also increases the sleeping time similar to thiopental.[14] The aim of the study was to evaluate the anxiolytic property of aqueous root extract of *Cymbopogon citratus* on behavioral and anxiety models in mice.

Materials and Methods

Plant material

Cymbopogon citratus was obtained from an herbal garden in Jos, Plateau State, Nigeria. The plant was identified by Ikechukwu Chijioke of Federal College of Forestry Jos. The root was washed and air-dried at 25 °C for two weeks, then crushed into coarse powder.

Plant extraction

Sixty grams of the powdered root was measured and dissolved in sufficient quantity of water for 48 h with mechanical shaking (4 h/day), at the end of 48 h; the mixture was filtered with ashless filter paper. The extract was concentrated using the rotary evaporator at a temperature of 4°C. The concentrate was heated over a water bath to obtain a solvent free extract which was later stored in the refrigerator at 4°C.

Phytochemical screening

Preliminary qualitative phytochemical screening of the crude aqueous root extract of *Cymbopogon citratus* was carried out using standard procedures as described by Trease and Evans.[15]

Experimental animals

Swiss albino mice of either sex (18-30 g) were obtained from the Animal House unit of University of Jos. The animals were given standard laboratory diet formulated by the Animal House Unit, Department of Pharmacology and water *ad libitum* and maintained under laboratory conditions i.e. temperature (22 ± 1°C), relative humidity (45-55%) and a non-reversed 12/12 h light-dark cycle.

Oral acute toxicity study

The modified Lorke's method,[16] was used in the LD_{50} test of the aqueous root extract of *Cymbopogon citratus*. This test was carried out in two phases. In the first phase, nine mice randomized into three groups of three animals per group, were administered with 10 mg/kg, 100 mg/kg and 1000 mg/kg of the extract orally. The mice were observed at the very first four hour for any behavioral sign of toxicity. The same procedure as used in first one was adopted in phase two but with different dose levels of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. LD_{50} was calculated using the formula:

$LD_{50} = \sqrt{}$ (lowest lethal dose × highest non lethal dose)[16]

Ethic

The Anxiolytic study was carried out to the "Principles of Laboratory Animal Care",[17] and in accordance to standard experimental procedure approved by the ethical Committee of Animal House, Department of Pharmacology University of Jos after filling of the ethic form.

Behavioral testing

The experiments were conducted in a room outside the breeding room between 9 h and 16 h. Data were recorded using a stop clock and manual counter. An independent group of mice was used for each behavioral test. The apparatus was cleaned with methylated spirit and cotton wool after each test. Mice were naive to the test apparatus.

Stress induced hyperthermia model

This experiment was carried out using the method described by Groenink.[18] Singly-housed mice were subjected to two measurements of body temperature, 10 min apart. The animals were singly housed 24 h prior to the experiment in a ventilated room to avoid possible effect of circadian rhythm on the body temperature; then grouped into five mice per group. Animal cages were taken to the experimental room and left for an hour prior to the experiment for acclimatization, with each group given 200 mg/kg, 400 mg/kg and 600 mg/kg of the aqueous root extract and 2.5 mg/kg of diazepam, while the normal saline group was given normal saline 5 ml/kg respectively. The mice were each picked up on the wire ceiling from its cage and gently immobilized by hand. An olive oil lubricated digital thermometer was used to measure the body temperature of the animals. The whole procedure (picking up of the animals, insertion of the probe, waiting for stabilization and releasing of the animal) was completed within 30 seconds. Body temperature of each mouse was measured one hour after oral administration of the extract at T_1 and then T_2 10 minutes later. The difference between temperature T_1 and T_2 was then calculated.

Elevated plus-maze model

The elevated plus-maze model was carried-out using the method described by Lister.[19] The elevated plus-maze consists of two open arms (25 × 10 cm each), and two closed arms (25 × 10 × 10 cm each), with an open roof. All four arms were

radiated from a central platform (10 × 10 cm). The maze is elevated to a height of 60 cm in a dimly lit room. Normal saline (10 ml/kg, orally), plant extract (200, 400 and 600 mg/kg, orally) and diazepam (2.5 mg/kg, orally) were administered to groups of 5 mice each. One hour post treatment, each mouse was placed in the center of the elevated plus-maze, facing one of the closed arms. During a 5 min test period the following parameters were taken: the number of entries and time spent in the open and enclosed arms. Entry into an arm was recorded when the mice cross the demarcation of respective arm with its four paws, and was considered to be on the central platform whenever two paws were on it.

Open field model

Each mouse was placed in a open-field apparatus (45 × 45 × 40 cm), made of wooden floor and glass sides. The floor was carved into 9 equal sized squares (15 × 15 cm). An hour before dropping the individual mice in one of the corner of the box (i.e. 60 min prior), the different groups were administered with respective treatments (Normal saline, diazepam 2.5 mg/kg, extract doses of 200 mg/kg, 400 mg/kg and 600 mg/kg) and then locomotion (number of both central and peripheral crossings), number of rearing and defecation were recorded for 5 min.

Statistical analysis

The mean ± S.E.M (standard error mean) was calculated from each group in each test. Statistical significance was determined by one-way ANOVA followed by Bonferroni post-test and values of $P < 0.05$ were considered significant. The analysis was performed using the instant graph pad prism (version 5.02)

Results

Phytochemical screening

Results obtained from the phytochemical screening of the aqueous root extracts of *Cymbopogon citratus* showed the presence of alkaloids, carbohydrates, tannins, flavonoids, cardiac glycosides, steroids, saponins and anthraquinones.

Oral acute toxicity test

No mortality was recorded in all experimental dose levels used in both phases, but mice showed some behavioral signs of toxicity at 5000 mg/kg. The LD_{50} of the aqueous extract was estimated to be greater than 5000 mg/kg.

Anxiolytic activity of aqueous root extract of Cymbopogon citratus on stress induced hyperthermia in mice

Difference in temperature (ΔT) decreased across the extract treated and diazepam group [Table 1]. The significant ($P < 0.01$) reduction in the temperature cut across the doses of 400, 600 mg/kg of the extract and 2.5 mg/kg diazepam with 0.73°C, 0.60°C and 0.33°C as ΔT. Similar effect was recorded at T_2 (final temperature) with decreased in the temperature in the extract treated and diazepam group. T_2 of 400, 600 mg/kg and 2.5 mg/kg of diazepam group were 34.70°C, 32.18°C and 34.60°C.

Anxiolytic activity of aqueous root extract of Cymbopogon citratus and diazepam on various parameters in elevated plus maze

Experimental doses of 200, 400 mg/kg extract and 2.5 mg/kg of diazepam produced significant ($P < 0.05$) increase in time spent in the open arms when compared to the normal saline group [Table 2]. But however, extract dose of 600 mg/kg had no significant ($P > 0.05$) effect. The duration of time spent in the open arm with diazepam treated group exceeded that of the control and the extract groups. Also, there was increase in the number of entries into the close and open arms.

Anxiolytic activity of aqueous root extract of Cymbopogon citratus various parameters in open field

The number of locomotion across the square in the open field increased within the extract treated and diazepam

Table 1: Effect of aqueous root extract of *Cymbopogon citratus* and diazepam on stress induced hyperthermia in mice

Treatment	T_1 (°C)	T_2 (°C)	ΔT (°C)
Normal Saline (5 ml/kg)	32.88 ± 0.22	34.60 ± 0.96	2.33 ± 0.50
200 mg/kg	32.85 ± 0.22	33.70 ± 0.35	1.10 ± 0.07
400 mg/kg	37.90 ± 0.18	34.93 ± 0.35	0.73 ± 0.18**
600 mg/kg	35.25 ± 0.23	34.70 ± 0.26	0.60 ± 0.21**
Diazepam (2.5 mg/kg)	32.40 ± 0.22	32.18 ± 0.14**	0.33 ± 0.19**

Values expressed as mean ± SEM; n – 5; **($P < 0.01$); T_2 (°C) – Initial temperature; T_2 (°C) – Final temperature; ΔT – Difference in temperature

Table 2: Effect of aqueous root extract of *Cymbopogon citratus* on elevated plus maze

Treatment	Time spent in seconds			Number of entries	
	Open arms	Closed arms	Center	Open arms	Closed arm
Normal Saline (5 mg/kg)	15.40 ± 2.14	235.80 ± 7.24	46.80 ± 8.15	4.40 ± 1.08	3.80 ± 0.86
200 mg/kg	60.00 ± 25.29*	191.20 ± 20.98*	44.60 ± 17.60	8.40 ± 1.60	4.80 ± 1.74
400 mg/kg	84.60 ± 11.86***	171.20 ± 14.49***	44.20 ± 6.77	6.40 ± 1.08	4.40 ± 0.60
600 mg/kg	48.00 ± 10.27	156.20 ± 17.00***	95.80 ± 7.08*	8.40 ± 1.20	5.40 ± 0.98
Diazepam 2.5 mg/kg	168.00 ± 24.89***	100.00 ± 19.32***	34.00 ± 7.66	5.20 ± 1.07	8.20 ± 1.39

Values expressed as mean ± SEM; n – 5; *($P < 0.05$); **($P < 0.01$); ***($P < 0.0001$)

group. The number of movement was well pronounced, significant ($P < 0.05$) at doses 200 and 600 mg/kg of the extract with values as 34.20 ± 15.09, 36.20 ± 9.04 while that of the control was 10.00 ± 6.12 [Table 3]. The number of rearing in the extract treated group decreased with increase recorded in the control group. Also, there was decreased in the number of defecation at 200, 400 mg/kg of the extract as well as the diazepam group with values as 1.20 ± 0.37, 1.50 ± 0.58 with least number of defecation at dose 600 mg/kg.

Discussion

Animals show behavioral response like anxiety when introduced to a novel environment. This could be a protective mechanism adopted by animals to get acclimatized to the environment. Some degree of anxiety is a part of normal life, but however, treatment is needed when it is higher than normal with respect to the situation.[20] The clinical use of benzodiazepines (like diazepam) in the treatment of anxiety disorders have been presented with limitations due to their adverse effects which includes psychomotor impairment, potentiating of other central depressant drugs and dependence.[9] This has prompted the search for a novel and potent anti-anxiety agents with fewer side effects. This study explores behavioral and anxiety models to evaluate the anxiolytic property of aqueous root extract of *Cymbopogon citratus*.

Stress induced hyperthermia (SIH) is a reliable, feasible and stable phenomenon found in many species including mice,[21,22] rat[23] and human. SIH test focus on the physiological component of stress and anxiety.[18] Expressions of increased heart rate, blood pressure and hyperthermia are good examples of symptoms of physiological hyper-arousal that are specific to ''anxiety''. The SIH response uses the transient rise in the body temperature in response to a stressor, the first body temperature measurement (T_1) is the basal unstressed core temperature but also functions as a stressor, whereas the second body temperature measurement (T_2) is the stress-induced body temperature which is increased due to the stress experienced from the first temperature measurement. The difference in the temperature ($\Delta T = T_2$-T_1) is defined as the SIH response. The extract caused a significant ($P < 0.05$) reduction in the body temperature (T_2 and ΔT) at experimental doses of 400, 600 mg/kg and diazepam 2.5 mg/kg when compared to the normal saline group which was higher. SIH is reduced by anti-stress drugs.[24,25] The effect of the extract on the body temperature was negated in a dose dependent fashion. SIH reductions were apparent in the diazepam and extract (400 mg/kg and 600 mg/kg) treated groups, but low dose of the extract (200 mg/kg) did not abolish the SIH response. The increase in ΔT in the normal saline group indicates SIH. The rise in the temperature in SIH is not caused by metabolic changes but a regulated process with physiological changes.[26,28] Importantly, the rise in the body temperature are accompanied by increase in the plasma adrenocorticotropic hormone (ACTH), corticosterone and glucose levels respectively,[28] more-so leading to the stimulation of the cardiovascular system,[27] and simultaneous mobilization of the brain and pituitary opioids.[29] The decreases in the body temperature (ΔT) of the extract treated group portray anxiolytic property of the extract.

Table 3: Effect of aqueous root extract of *Cymbopogon citratus* and diazepam on open field

Treatment	No. of locomotion	No. of rearing	No. defecation
Normal saline (5 mg/kg)	10.00±6.12	5.60±0.93	1.70±0.32
200 mg/kg	34.20±15.09**	4.60±1.33	1.20±0.37
400 mg/kg	24.80±8.79	2.80±0.49	1.50±0.58
600 mg/kg	36.20±9.04**	4.00±0.55	0.60±0.40
Diazepam (2.5 mg/kg)	23.40±2.16	5.40±0.60	1.20±0.58

Values expressed as mean ± SEM; n – 5; **($P < 0.01$)

Elevated plus maze is a simple and widely used model to measure anxiety like behavioral pattern in rodents.[30] Animals develop anxiety-like behavior when placed on the elevated plus maze due the fear of height.[31] In the EPM, extract doses of 200 mg/kg and 400 mg/kg and 2.5 mg/kg of diazepam significantly ($P < 0.05$) increased time spent by the mice in open arms of the elevated plus maze, with diazepam group having the highest anxiolytic effect followed by extract treated group in a non dose dependent manner. They were also increase in the number of entries into the closed and open arms of the elevated plus maze. Increase in the motor activity is a feature of agents with anxiolytic property, which is measured by the time spent by the animal in the open arms of elevated plus maze.[32] Increase in time spent in the open arm entry parameters are the most representative features of anxiolytic agents.[33] Agent with anxiolytic property decreased anxiety by increasing the open arm exploration.[32] However, 2.5 mg/kg of diazepam caused an increase in the time spent in the open arms of the elevated plus maze which was much higher in comparison to extract treated and normal saline group. In the normal saline group, the spent time in the closed arm exceeded that of the open arm. Increase in time spent in the closed arms of the maze is an important induces of anxiety which is characterized by decrease in motor activity.[31] The period of time spent on the central platform appears to be related to decision making and the number of arm entries is a contaminated measure reflecting changes in anxiety and general activity. This portrays a manifestation of anxiety.[34]

The open field model examines related psychomotor performance, normal aversion and behavioral aspect of

rodent when placed in open brightly lit area. Animals express some form of fear (sympathetic arousal) and anxiety (avoidance, muscle tension) when removed from their acclimatized cage by showing alteration in all or some parameters,[35] due to thoughts of imminent threat. The locomotive activity of the mice significantly ($P < 0.05$) increased at extract doses of 200 mg/kg and 600 mg/kg across the square of the open field, higher at these doses of the extract compared to diazepam and the normal saline group. This increase clearly demonstrates anti-anxiety effect of the extract. The extract may be acting similarly as the standard drug. The number of rearing was less in the extract treated groups with corresponding reduction in defecation. The reduction of rearing and defecation induced by the extract in elevated plus maze and open field models are indices of anxiolytic properties.[36] Anxiolytic property of the extract may be linked to the active principles present in it. Flavonoids, alkaloids, terpenoids have been found to possess anxiety property in different plant extracts.[37]

The exact mechanism by which the extract exhibited its anxiolytic effect cannot be ascertained, but one may likely suggest that the extract mimic the anti-anxiety effect of the standard diazepam, a class of benzodiazepine which act through the $GABA_A$ receptors. Different neurochemical metabolisms are involved in brain activation, dopamine (DA) appears to play an essential role. It is generally believed that locomotor activation results from brain activation, which manifests as excitation of central neurons and as san increase in cerebral metabolism. Increase in the locomotor activity of the mice could be as a result of increased excitability of central nervous system.

Conclusion

In summary this study provides a broad spectrum for the exploration of the Anxiolytic property of *Cymbopogon citratus* extract. However, further study is needed to identify the exact active principle and probably to ascertain the mechanism of action of anti-anxiety at molecular level.

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