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Evaluation of Anxiolytic Activity of Ethanolic Extract of *Thymus vulgaris* Leaves Using Elevated Plus Maze [EPM] Test

Modupe Iretiola Builders [1*], Rachel Abimbola Oyefeso[2], Samuel Otokpa Ede[3], Simeon Oyepata Joseph[4], Peter Uduak Ise[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Bingham University, Karu, Nasarawa State, Nigeria
[2]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Jos, Plateau State, Nigeria
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Federal University, Oye-Ekiti, Ekiti State, Nigeria

Corresponding Author
Email Id: modupebuilders@yahoo.com

ABSTRACT

The most common group of psychiatric diseases are anxiety disorders, thus there is increase need for anxiolytics as a result of increasingly paced world. The current anxiolytics are characterized by serious adverse effects and narrow therapeutic margin, therefore, there is need for alternative drugs in terms of plants which are cheaper, more accessible and more affordable. Thymus vulgaris L. [Lamiaceae] is a medicinal plant belonging to the Lamiaceae family, Ethinomedicinally T. vulgaris has anti-anxiety, sedative, hypnotic antihelminthic, expectorant, antiseptic, antispasmodic, antimicrobial, antifungal, antioxidant, antivirotic, carminative, and diaphoretic effects. The anxiolytic activity of T.vulgaris L was evaluated using three different doses [50mg/kg, 100mg/kg and 200mg/kg body weight of the extract], 5mg/kg diazepam was also used a reference drug by EPM. The result indicated statistically dose-dependent reduction of anxiety activities [P<0.05] and highly statistically dose-dependent reduction of anxiety activities [P>0.05] in EPM test. The phytochemicals responsible for the anti-anxiety effects should be elucidated and characterized.

Keywords*: Anxiolytic Activity, Thymus Vulgaris Leaves, Elevated Plus Maze [EPM] Test.*

INTRODUCTION

Anxiety is a mental disorder which as a result of continuous state of restlessness in patients, this disorder is similar to other mental disorders such as panic attacks and psychosis and can also be categorized into simple and chronic anxiety [1]. Anxiety is differentiated from other disorders such as fear which is an emotional response while anxiety is a normal response [2]. The major consequences of this disorder are personal adverse effects as well as societal adverse effects [3], the causes of this psychiatric disease include physical conditions such as diabetes mellitus, chronic disorders such as depression, hereditary diseases and environmental factors such drug abuse as well as other miscellaneous conditions such as stress [4].

The most commonly used drugs to treat this psychiatric ailment are benzodiazepines, however, benzodiazepines have narrow therapeutic index warranting serious unwanted side effects. Thus, there is need to search for a novel anxiolytic drug with fewer undesirable effects [5].

Plants which are also known as complementary medicines have been used from time memorial for managing various illnesses because they are readily and cheaply available healthcare alternatives [6]. These medicinal plants are used all over the world because of their medicinal values as well as food spices and food flavorants, they are available in form of crudes, extracts, fractions and dry powder. They are medicinal and aromatic plants for development and preparation of alternative traditional medicine and food additives [7].

Thyme which is scientifically called [*Thymus vulgaris* L.], belongs to Lamiaceae family of Labiatae of the plant kingdom. It is a family of flowering plants with aroma. Basil, rosemary, savory, oregano, sage, lavender, mentha, mint, perilla are other plants of the same species, including thyme [8]. Thyme possesses many medicinal uses which include anti-anxiety, sedative, hypnotic, antihypertensive, anticancer, analgesics, antibacterial, antifungal and antioxidants. The bioactive components of *T.vulgaris* L are essential oil, saponins, tannins, steroids, flavonoids, these phytochemicals are attributes to the pharmacological activities of this medicinal plant [9].

There are variety of animal models for evaluating the anxiolytic activities of drugs, however, the most commonly used test is elevated plus maze [EPZ], it is an established animal test causing a fear status by comprehensible stimuli and the use of innate behavior of animals and is a validated and also reliable test for detecting both anxiolytic- and anxiogenic-like effects of agents [10]. Since majority of the anxiolytics are associated with serious adverse effects due to narrow therapeutic index and several researches have shown that natural anxiolytic drugs have therapeutic relevance in the treatment of anxiety, therefore, this study was carried out to assess the anxiolytic potential of *T.vulgaris* L .

MATERIALS AND METHODS

Chemicals And Reagents

Ethanol [BDH limited poole, England], Normal saline [N/S], distilled water, diazepam, Fabricated elevated plus maze, Centrifuge, measuring cylinder, mortar and pestle, separating funnel, beakers, cotton wool, retort stand, PH meter, rotary Evaporator and soxhlet apparatus.

Animals

Male Wistar rats [230-250 g]

Experimental Animals

Male Wistar rats, weighing 230-250 g, were allowed to acclimatized for 72 hours prior to the test. They were housed in groups of six per cage under a 12:12 dark/light cycle [lights on at 07:00 AM] at 22 ± 2^0C and given free access to food and water. Rats were randomly assigned to different treatment groups [n=5]. Animals were tested under the same experimental conditions. All experiments were carried out in a quiet room under controlled light conditions between 11:00 AM and 3:00 PM. Behavioral observations were conducted in quiet rooms at the same period of the day to reduce the confounding influence of diurnal variation on spontaneous behavior. Each animal was tested only once [11].

Ethical Approval

All research and animal care procedures were approved by the Bingham University Research Ethics Committee and were performed in accordance with international standards of animal welfare recommended by the Society for Neuroscience [Handbook for the Use of

Animals in Neuroscience Research, 1997]. The minimum number of animals and the minimum duration of observation required to obtain consistent data were used [12, 13].

Grouping Of Animals and Dosing

Thirty [30] female wistar albino rats of 230-250g were randomly divided into five different groups of six rats in each group. The negative control [NC] group was given distilled water [GROUP 1]. Treatment groups II, III and IV were given T.*vulgaris* L ethanolic extract 50mg/kg, 100mg/kg and 200mg/kg respectively. Group V was treated with 5mg/kg diazepam.

Collection, Preparation and Extraction of Plant Material

Leaves of *T. vulgaris* were collected in spring. The plant material was dried at 40^0C with air circulation, ground, and extracted with ethanol by percolation at room temperature. The extract was then taken to the laboratory for the process of evaporation. The evaporation process involved complete removal of ethanol and water used for the extraction. The extracts were dried at 40^0C under vacuum and finally freeze dried. Pharmacological assays were carried out with aqueous suspensions of the dried extract. The doses were expressed as milligrams of dried extract per kilogram of rat body weight. The extracts were dissolved in their solvents prior to each individual experiment.

EPM TEST

The EPM was fabricated, composition of black aluminum and two open, elevated [51 cm from the floor] arms and two enclosed arms and together they built a cross as indicated in Figure 1b. Each arm is 40 cm long and 10 cm wide and the walls are 40 cm high. The center of the maze [10 x 10 cm] was not considered to be either a closed or open space. The rat was placed in the center with the nose pointing at one open arm. Each trial was 10 minutes and after each trial the maze was cleaned with 10 % ethanol solution. The lightning conditions [1x] were 100 at the open arms [14].

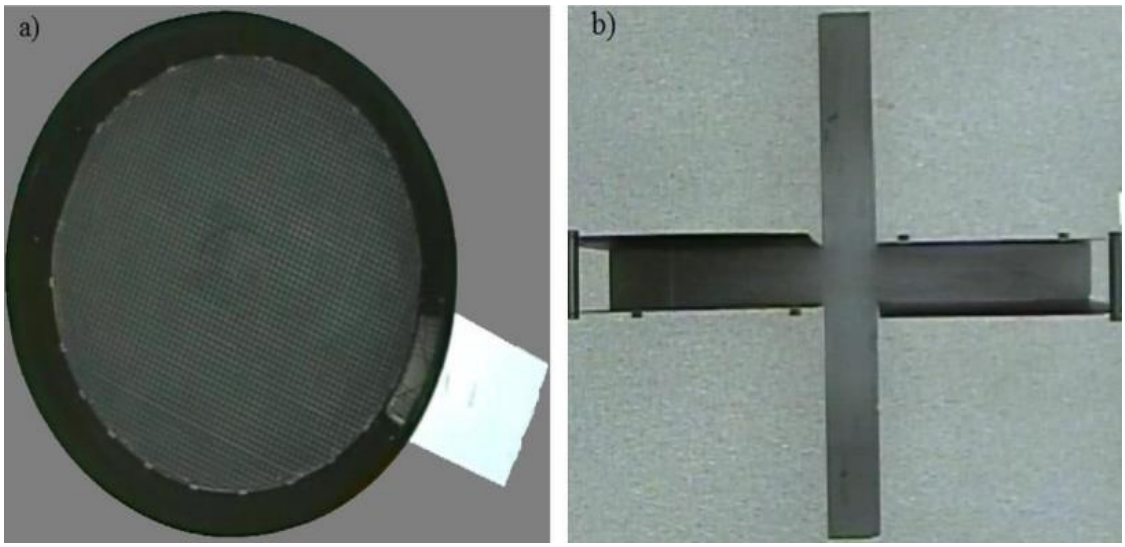

Fig. 1[a] The open field test with a start box attached and [b] the elevated plus maze.

Statistical Analysis

Results were expressed as mean standard error of the mean. The difference between the mean was determined by one-way analysis of variance [ANOVA]. In all cases, differences were

considered significant if less than 0.05 [P<0.05] and highly significant if greater than [P> 0.05].

RESULTS AND DISCUSSION

Table 1 : Anxiolytic Activities of Ethanolic Extract of *T.Vulgaris* Leaves in Rats by Elevated Plus Maze [EPM] Test

Extract/Drug [Mg/Kg]]	Percentage Entries [%]	Time Spent [S]	Number of Entries	Total Distance [Cm]
Distilled water [5ml]	42.2 ± 0.05	117.5 ± 0.22	17.7± 0.41	1180 ± 0.33
50	48.1 ± 0.15*	124.0 ± 0.10*	15.0± 1.00	1170 ± 1.20
100	57.9 ± 1.00**	138.2 ± 0.68**	18.2 ± 0.03	1170 ± 1.01
200	68.9 ± 1.02**	168.1 ± 1.00**	17.8 ± 0.99	1180 ± 0.78
DIAZEPAM [5mg]	67.8 ± 0.32**	167.5 ± 1.15**	17.0 ± 1.11	1170 ± 0.01

*Values are represented as mean ± S.E. M, n=6, *P<0.05, significantly, *P>0.05, highly significant different from control [Distilled water].*

Anxiolytic Activities of Ethanolic Extract of *T. Vulgaris* Leaves

The ethanolic extract of *T.vulgaris* leaves displayed both significant [P< 0.05] and highly significant [P> 0.05] dose-dependent anxiolytic activities as illustrated in percentage entries, time spent, number of entries and total distance as indicated in table 1.

Many plants interact with the central nervous system receptors to produce their pharmacological effects and are used to treat chronic conditions such as anxiety and depression that do not respond well to conventional therapeutic treatments [15]. Various types of drugs have been discovered to produce anxiolytic effect, thereby, have been used as anxiolytics in different part of the world [16].

In this study, four parameters (percentage entries, time spent, number of entries and total distance) were assessed in EPM model. The elevated plus maze [EPM] test is a widely used behavioral assay for rodents and has been validated to assess the anti-anxiety effects of pharmacological drugs and steroid hormones, and to define brain regions and mechanisms underlying anxiety-related behavior.

Rats are placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm are recorded and observed simultaneously for 10 min. other ethological parameters (*i.e.*, rears, head dips and stretched-attend postures) can also be observed. An increase in open arm activity [duration/entries] reflects anti-anxiety behavior. The elevated plus maze has been described as a simple method for assessing anxiety responses of rodents [10, 17] and can also be used to evaluate simple anxiety.

Handley and Mithani described the assessment of anxiety behavior of rodents by using the ratio of time spent on the open arms to the ratio of time spent on the closed arms. Unlike other behavioral assays used to assess anxiety responses that rely upon the presentation of noxious stimuli [i.e., electric shock, food/water deprivation, loud noises, exposure to predator odor, etc.] that typically produce a conditioned response, the elevated plus maze relies upon

rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces [avoidance] [14].

Our findings indicated that the extract of *T.vulgaris* increased the percentage of both entries and time spent in the Elevated plus maze. Therefore, the extract was able to produce anxiolytic effect in the rats after 1-week of oral administration. The effect of *T. vulgaris* was not induced by changes in motor activity at these doses, because the total distance covered by the rats was not altered. An increase in the time and proportion of the entries into the open field maze lacking a changed locomotor activity is confirmed as a potent sign of anxiolytic substance effect. *T. vulgaris* [18].

T. vulgaris is used mainly as food seasoning, but also pharmaceutically as a source of essential oil that are used in perfumery and also as a worming and antibacterial drug. Thyme is also known to contain a high concentration of phenolic compounds such as thymol and carvacol, which are essential oils. Wild thyme contains phenolic compounds with caffeic acid and rosmarinic acid derivatives being the most important of these. Reports show that the volatile oil of thyme are among the main essential oils used in the food industry and in cosmetics as preservatives and antioxidants.

T. vulgaris consists of a mixture of monoterpenes. The main compounds of this oil are the natural terpenoid thymol and its phenol isomer carvacol, which have anxiolytic, sedative, hypnotic, antioxidant, antimicrobial, antitussive, expectorant and antispasmodic [8].

Research has shown that ethanol thyme extract can be used as a natural antioxidant to prolong the stability of oils, which are responsible for anti-anxiety activities of the extract and may be effective in treatment of chronic anxiety [19]. It could also be concluded that the essential oil of *T. vulgaris* has a potential anxiolytic activity which is dose- dependent. Extensive research has been conducted to reveal multiple neural substrates and mechanisms that contribute to the cause of depression and anxiety, among which the imbalance between oxidation and the antioxidant defense system has gained attention.

Some studies have further demonstrated the role of oxidative stress in anxiety of rodents. Furthermore, it has been reported that two drugs that induce oxidative stress, buthionine-[S,R]-sulfoximine and xanthine plus xanthine oxidase (X+XO} cause increased anxiety-like behavior in rats [20]. In the present study, *T. vulgaris* extract was found to decrease the level of anxiety in animals. *T. vulgaris* oils contains bioactive monoterpenes such as linalool. Recent findings show that linalool inhalation reduces anxiety [21].

The presence of linalool and linalyl acetate in plant extract supports the claim that the extract produces sedative effects [21]. In another recent study, it has been shown that kaempferol induces anxiolytic activities in the EPM test in mice [22]. It has also been shown that carvacol presents anxiolytic effects in the plus maze test [22], which are not influenced by the locomotor activity in the open field test. It is possible that these components play essential roles in the anxiolytic properties of *T. vulgaris* in the EPM test [21].

CONCLUSION

The presence of polyphenols, flavonoids, and essential oil in the extract of *Thymus vulgaris* may be attributed to the anxiolytic effects and was found to be dose-dependent. Further study should be carried out to determine the exact mechanism of action responsible for the anxiolytic effects.

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