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Anxiolytic Testing of Medicinal Plants in Nigeria: Frequently Used Experimental Models

Nijerya'daki Tıbbi Bitkilerin Anksiyolitik Etkisinin Test Edilmesi: Sıkça Kullanılan Deneysel Modeller

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ABSTRACT

Psychopharmacology, especially behavioral studies is attracting increasing interest of researchers because of lower quality of life, and higher prevalence of mental disorders such as anxiety in Nigeria. Various experimental animal models have been used successfully to demonstrate the anxiolytic property of medicinal plants. Techniques such as open field test, elevated plus maze, staircase test method, light, and dark box test, hole-board test, and beam walking assay are available and functioning effectively in various pharmaceutical research centers and higher education institutions in Nigeria. Consequently, this has led to the advancement in the field of behavioral studies. Furthermore, these experimental models are easy to operate, and in many instances, yielded promising and reproducible results. However, the accuracy and the validity of the outcome depend on the experience of the researcher, familiarization with laboratory animals and in-depth knowledge of animal psychology. It is recommended that experimental models of anxiolytic testing can be improved by making an automated apparatus connected to digital watches, video cameras and computers available in Nigeria. The primary goal of this paper is to discuss the most commonly available experimental models in the evaluation of the anxiolytic activity of medicinal plants in Nigeria and to give a recommendation for further improvement and drug development.

Keywords: *Anxiolytic, open-field, elevated-plus-maze, rodents, medicinal-plants*

ÖZ

Psikofarmokoloji, özellikle davranışsal çalışmalar, düşük yaşam kalitesi ve anksiyete gibi mental bozuklukların yüksek prevelansı nedeniyle Nijerya'da giderek daha fazla ilgi gören bir farmakoloji alanıdır. Tıbbi bitkilerin anksiyolitik etkisini göstermede laboratuvar hayvanları kullanılarak çeşitli deneysel modeller başarı ile kullanılmıştır. Açık alan testi (open field test), yükseltilmiş artı labirent testi (elevated plus maze), merdiven testi (staircase test method), aydınlık karanlık kutusu testi (light and dark box test), delikli kutu testi (hole-board test) ve denge ışın yürüme testi (beam walking assay) gibi teknikler mevcut olup, Nijerya'da çeşitli farmasötik araştırma merkezleri ve yüksek öğretim kurumlarında etkili bir şekilde işlev görmektedir. Sonuç olarak, bu durum davranışsal çalışmalar alanında ilerlemelere öncülük etmiştir. Üstelik bu deneysel modeller yapılması kolay olup, birçok durumda güvenilir ve tekrarlanabilir sonuçlar ortaya çıkarmıştır. Ancak sonuçların doğruluğu ve geçerliliği araştırmacının deneyimine, laboratuvar hayvanlarını ne kadar yakından tanıdığına ve hayvan psikolojisi hakkında geniş kapsamlı bilgisi olmasına bağlıdır. Anksiyolitik etkinin test edilmesindeki deneysel modellerin, Nijerya'daki mevcut dijital kol saatleri, video kameralar ve bilgisayarlar ile bağlantı kuran bir otomatik cihazın yapılması ile geliştirilebileceği öne sürülmektedir. Bu makalenin primer amacı, Nijerya'daki tıbbi bitkilerin anksiyolitik aktivitesini değerlendirmede en sık kullanılan mevcut deneysel modelleri tartışmak ve daha ileriye gitme ve ilaç geliştirilmesine yönelik bir öneride bulunmaktır.

Anahtar kelimeler: *Anksiyolitik, açık alan, yükseltilmiş artı labirent, kemirgenler, tıbbi bitkiler*

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INTRODUCTION

Globally, there is a renewal of interest in the search and consumption of indigenous medicine, especially for mental and neurological disorders. Several countries in the West, Europe, and Asia have gone far in the pursuit of knowledge and practice of traditional medicine. Studies of natural medication around the world include Ayurvedic medicine, Unani medicine, Kampo medicine, Acupuncture, and Chinese oriental medicine, etc.[1,2]. Worldwide, mental and neurological disorders account for up to 13% of the global burden of diseases. In addition, government legislation on mental health is very poor especially among the developing nations[3-5]. Also, the number of mental health specialist is grossly inadequate with about 1 psychiatrist per 200,000 people or more. Furthermore, among developing countries, only 36% of the people with mental disorders are covered by mental health legislation but up to 92% in developed nations[3-5]. The cost of treating mental disorders is the major obstacle in decreasing the welfare of the patient. As such, up to 35% to 50% of patients with severe mental disorders in high-income countries do not receive treatment. Unfortunately, these figures rose to 76% to 85% in low-income countries[3-5]. In Nigeria, especially in the rural areas, people do not have easy access to the anxiolytic drugs; even if available they may not be affordable by the local people. In addition, anxiolytic drugs are either poisons or prescription-only medicine that involves lengthy procedures before they are dispensed to the patient[5-7]. Anxiety also known as social phobia, is characterized by a significant amount of fear in one or more social situations, causing considerable distress and impaired ability to function in at least some parts of daily life. Physical symptoms often include excessive blushing, sweating, trembling, palpitations, and nausea. Stammering may be present, along with rapid speech; panic attacks may occur under intense fear and discomfort[8,9]. Anxiety disorders are usually treated with benzodiazepines, selective serotonin reuptake inhibitors, tricyclic antidepressants or serotonin-norepinephrine reuptake inhibitors which calm and relax the patient and reduce anxiety. Unfortunately, despite their therapeutic effects, they cause numerous side effects like dizziness, drowsiness, cognitive impairment, physical dependence, psychological dependence and possible tolerance[10]. To minimize the above side effects, more researches should be conducted on medicinal plants with the hope of finding a safer and cost-effective alternative to the existing treatment modalities[1,2,11,12].

Several experimental models have been used in anxiolytic testing of medicinal plants or drugs under investigation using laboratory animals. Some of these models were used only for anxiolytic testing, some for sedative testing while others have dual purposes. The commonly used experimental models for anxiolytic testing in Nigeria include open field test (OFT), elevated plus maze (EPM), staircase test method (STM), light and dark box test (LDBT), hole-board test (HBT), and beam walking assay (BWA). The purpose of this review was to discuss experimental models commonly employed in testing of anxiolytic activity of medicinal plants in Nigeria and their advantages and disadvantages in behavioral researches.

SIX DIFFERENT ANXIETY TEST MODELS COMMONLY USED IN NIGERIA

OPEN FIELD TEST (OFT)

Open field test was first invented by Calvin Hall in 1934. The apparatus is used to measure defecation as a sign of emotionality, apprehension, and timidity. The technique describes the animal behaviors in a state of anxiety. Nonetheless, the interpretation of the results can be affected or modified by genetic factors, physiological changes, and pharmacological manipulations[13-15]. Open field test is employed to assess both anxiolytic and sedative property of extracts of medicinal plants or isolated compounds as well as their effects on locomotor activity in laboratory animals. Rodents placed in a new environment may experience signs of anxiety such as decreased mobility, exploration, grooming and rearing with concurrent increased urination and defecation[13,14,16,18].

Principle: Open field apparatus operates based on

Plate 1. Picture of an open field apparatus taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

the rodents' natural aversion for open field and enthusiasm to explore their environment for food, water and shelter[13,15,18].

OPEN FIELD EXPERIMENTATION

Apparatus: Open field apparatus consists of a clear flexible-glass (72 cm x 72 cm wide, 36 cm high) with white formica floor. The floor is divided into 16 squares of equal size (18 cm x 18 cm) using a red marker. A central square is drawn using a blue marker which is an intersection of four neighboring squares. The glass used is transparent so that both the animal and the central square can be viewed from the side[13,16,17,19,21]. Central square is preferred to start the testing, because it has a wide space from the surrounding walls as such several animals' behavior could be observed. Secondly, some species of rodents have a high level of locomotion. The dimensions of the testing room is usually 1.8 m x 4.6 m and illuminated with a 60-watt red lamp. A video camera connected to a computer screen is positioned 2 m above the apparatus[13,16,17,19,21].

Method of Testing: The initial step begins with habituation of the animals 20 minutes before the main test. Each animal is removed from the cage and placed inside an open field arena and allowed to habituate for 2 minutes. If the animals are newly supplied, they should be kept in their cage for 30 minutes to acclimatize[13,16,17,21]. The next step is the administration of an extract to the test groups, distilled water to the negative control group and standard drug to the positive control group. Thirty minutes later, the camera is on, the rodent placed at the central square of the apparatus and its behavior exhibited within 5 minutes is recorded. The apparatus is cleaned with 70% ethanol to remove urine and feces and prevent olfactory cue that is the ability of the rodents to follow the footsteps, urination or feces pattern of the preceding animal. This procedure is repeated with all the animals until all groups participated in the experiment[13,16,17,19,21].

Data Recording: The video is played, each parameter is observed independently and recorded. These include latency period, entry, and stay in the central square entry, and frequency of rearing[17,19,21]. In addition, other parameters like grooming, freezing, defecation, and urination are observed and recorded. The video may be played repeatedly to ensure accurate data recording[17,21,22].

INTERPRETATION OF OPEN FIELD RESULT

Latency Period: The longer the time spent by the animal, the more anxious they are. Decrease in latency period in an animal that was given an extract compared to the negative control group is an indication of the anxiolytic effect of the medicinal plant or drug under investigation[13,19,21,23].

Frequency of Rearing: Increase in the number of rearing, and line crossing signifies an increase in locomotion and exploration but low anxiety. Increase in number of rearing by animals that were given extract compared to the negative control group signifies anxiolytic property of the medicinal plant or drug under investigation[13,19,21,23].

Central Square Entry: Increase in the frequency of entries into the central square is an indication of low anxiety. Therefore, animals that are given plant extract which exhibit increase in frequency of entries into the central square reveal anxiolytic property of the medicinal plant or drug under investigation[13,19,21,23].

Stay in Central Square: Decrease in the duration of the stay in the central square reflects an anxiety state. Increase in duration of stay in the central square by animals that were given plant extract reveal anxiolytic property of the medicinal plant or drug under investigation[13,19-21,23].

Freezing: Animal placed in an open field apparatus may freeze and refuse to move away from the initial position which is an indication of the high level of anxiety. However, animals that were given plant extract, placed in an open field without freezing signifies the possibility of its anxiolytic activity[13,21,23].

Grooming: Rodent placed in a new environment may exhibit behavioral changes such as licking and washing face known as grooming. This behavior is an indication of anxiety state which may disappear whenever the rodent becomes adapted to the environment. Accordingly, rodents that were given plant extract and exhibit decrease in grooming show anxiolytic property of the extract[13,21,23].

Defecation and Urination: Frequent defecation and urination by an animal placed in an open field environment is an indication of emotionality and timidity. Thus, animals that were given plant extract that exhibit a decrease in defecation and urination implies anxiolytic activity of the medicinal plant or drug under investigation[13,23].

ELEVATED PLUS MAZE (EPM)

Elevated plus maze test is used to study rodents' behavior in an anxiety state and to determine the anxiolytic action of medicinal plants. This was observed as the ability of treated rodents to alleviate the fear of open space[24,29]. In addition, this technique can be used to study the functions of various part of the brain such as the hippocampus, limbic region, amygdala, and raphe nucleus. Initially, the apparatus was invented with Y-shape but later modified to plus (+) shape consisting of two open arms and two closed arms[22,28,29].

Principle: This test is carried out based on the conflict between rodents' natural aversion for high open space and curiosity to explore the environment for food, water and shelter[24,28].

PARAMETERS RECORDED

Latency Period: The time taken before making the first move is known as the latency period. In this experiment, the latency period should not exceed 30% of the total experiment time. Nonetheless, it is not frequently recorded in an elevated plus maze test[22,24,26,28].

Frequency of Open Arm Entry: Animal placed in elevated plus maze may enter open arm which shows the ability to alleviate the fear of open space. The number of times the animal entered the open arm is recorded as frequency of open arm entry at the end of 5 minute-experiment[22,24,26,28].

Open Arm Duration: The duration of open arm stay is recorded using a stopwatch any time the animal enters until it moves out. The time spent is finally summed to get the total duration of stay in an open arm[22,22,26,28].

Frequency of Closed Arm Entry: Anytime the rodents entered closed arm it should be tallied until the end of 5 minutes test. The total number of entry is later added to obtain the frequency of closed arm entry [22,24,26,28].

Closed Arm Duration: A stopwatch is pressed anytime the animal enters the closed arm until it moves out. The timing continues until the end of 5 minutes experiment. The total time of stay in closed arm is now added to get the duration of stay in closed arm[22,24,26,28].

ELEVATED PLUS MAZE EXPERIMENTATION

Apparatus: Elevated Plus Maze apparatus is made up of the wooden board comprising of two open arms (30 cm x 5 cm) without walls and two closed arms (30 cm x 5 cm x 15 cm) with walls. The two arms are connected through a central platform which is (5cm x 5cm). The apparatus has a plastic, or wooden support at the base placed 50 cm above ground level[22,24,27,29]. A video camera connected to a computer monitor for recording is stationed 2 m above the apparatus. Also, a light source that produces illumination of 2800 lumens is positioned at the ceiling above the apparatus[22].

Method of Testing: Rodents to be experimented are first kept in the testing room for 30 minutes to acclimatize. Subsequently, they are placed individually inside the apparatus 20 minutes before the experiment to habituate. Animals were given graded doses of plant extract, distilled water to the negative control group and standard drug to the positive control group[24-29]. Thirty minutes later the video camera is on; animals are placed individually at the center of the maze that is the intersection of four arms with the head facing the open arm. The animal behavior is observed and recorded for 5 minutes. The animal is considered to enter or leave an arm if it uses all of its 4 paws. The apparatus should be cleaned with 70% ethanol between tests to prevent olfactory cue[24-29]. The position of an observer should be in such a way

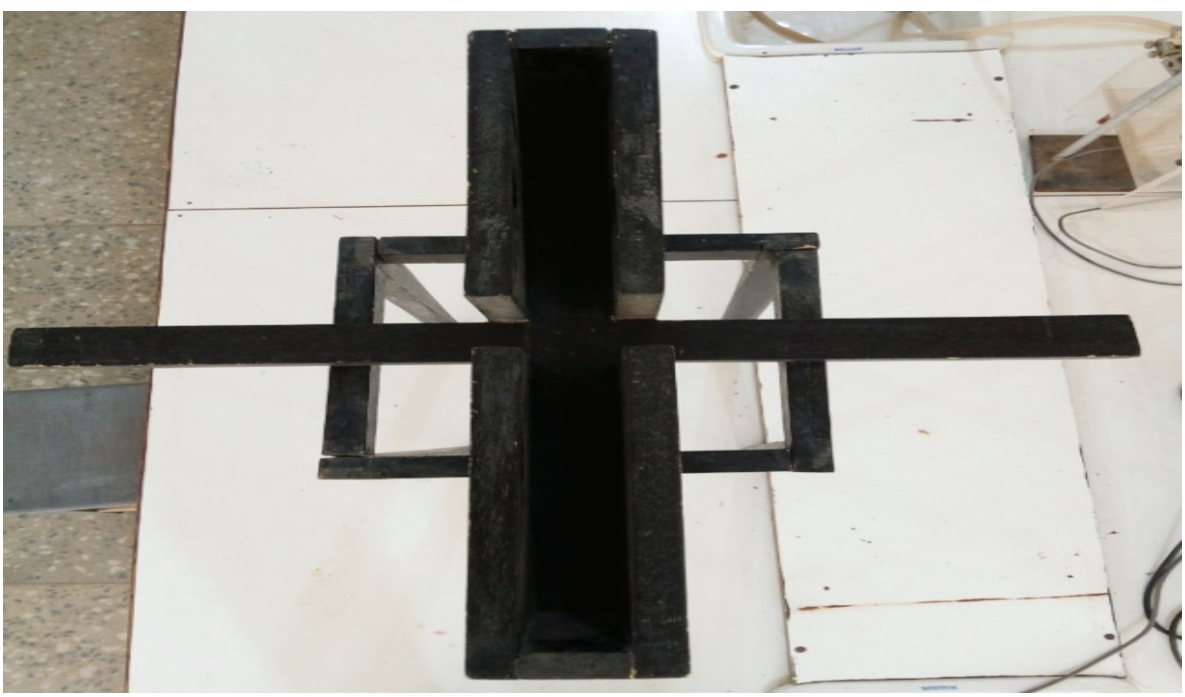

Plate 2. Picture of an elevated plus maze apparatus taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

that will not alter the animal behavior. An animal that falls during the test should be placed back and urged to continue the test but it should not be included in the analysis. Similarly, if the animal freezes and refuses to move completely, it shall remain in the experiment unless the freezing time exceedes 30% of the total testing time that is 100 seconds[22,25,26].

Data Recording: The video recorded can be played later to record the data concerning latency period, number of open arm entry, open arm duration, number of closed arm entry, and closed arm duration. All the parameters are monitored and recorded until 5 minutes of the experiment have elapsed[22,24,28-31]. The recording can be played multiple times to ensure accurate data reading. However, in a situation where there is no camera, the data can be obtained by using tally and stopwatch under careful observation.

INTERPRETATION OF ELEVATED PLUS MAZE RESULT

Latency Period: Increase in the time taken by animals before the first move in elevated plus maze apparatus is an indication of anxiety state. Conversely, decrease in the latency time before the first move by the rodents implies anxiolytic property of the medicinal plant or drug under test[22,24,25,28-31].

The Frequency of Open Arm Entry: Increase in the number entry into the open arm by the group of animals that were given plant extract is an indication of anxiolytic property of the extract. However, decrease in the frequency of entry into the open arm implies its anxiogenic activity[22,24,25,28-31].

Duration of Stay in Open Arms: In this test, increase in the duration of stay in open arms by the rodents that were given plant extract signifies anxiolytic activity of the extract. However, decrease in the duration of stay in an open arm implies an anxiety state[22,24,25,28-31].

Frequency of Entries into Closed Arm Position: Rodents in anxiety state may frequently move into the closed arm due to the natural desire for protection. Nonetheless, decrease in the duration of stay in closed arms signifies animal's ability to alleviate fear (anti-anxiety property)[22,24,25,28-31].

Duration of stay in Closed Arm: An increase in the duration of stay in closed arm reveals an anxiety state. However, the decrease in the duration of stay in closed arm by the animals that were given extract indicates anxiolytic activity of the medicinal plant or drug under investigation[22,24,25,28-31].

STAIRCASE METHOD

This experiment is primarily designed to test for both anxiolytic and sedative properties of medicinal plants, steroids, and other compounds. It is also carried out to study motor coordination deficit as well as the presence of a lesion in corpus striatum and substantia nigra[32-35].

PARAMETERS RECORDED

Number of Upward Step Climbed: During the experiment, the number of stairs climbed by the rodents is counted and tallied. A rodent is considered to have climbed a step when it places all of its 4 paws on it. At the end of the experiment the tallies were summed up to get the total number of upward steps climbed[26,31-35].

Frequency of Rearing: Rearing is counted whenever animal stood with hind legs and raised its front paws on air. The counting begins immediately the animal is placed inside the arena until the end of 5 minutes period[26,31-35].

Time Taken to Reach the Top: An experimental animal tends to move fast and reach the top stairs to explore the apparatus. The time taken by the animal to reach the top is determinant of animals' behavior and indication of fitness[26,31-35].

STAIRCASE METHOD EXPERIMENTATION

Apparatus: The apparatus used in this test comprises

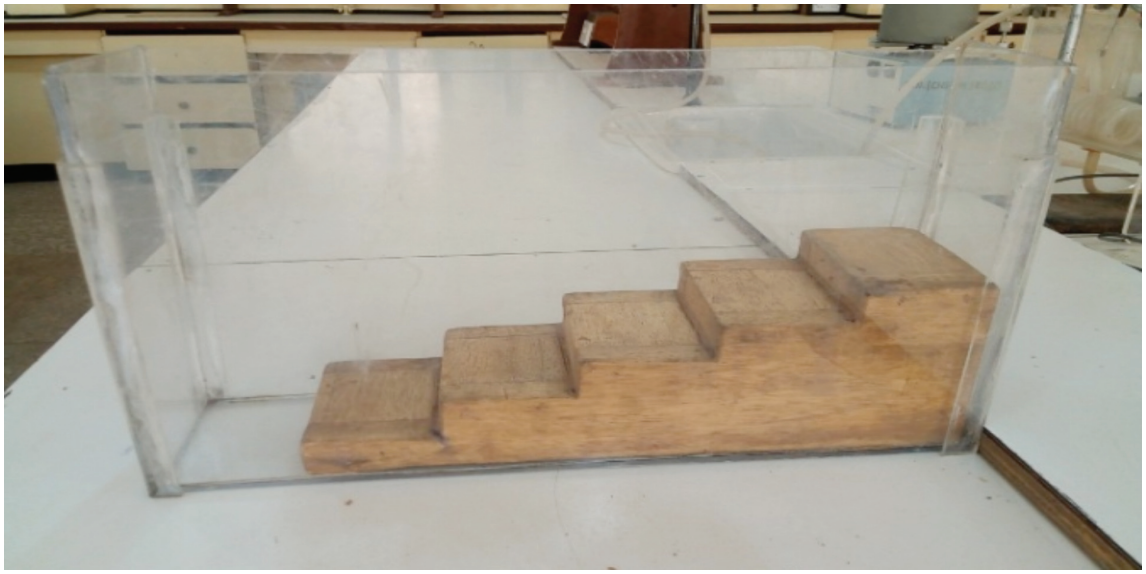

Plate 3: Picture of staircase test apparatus taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

of a wooden staircase enclosed in transparent Perspex vertical walls (45 cm x 12 cm x 25 cm). A removable staircase with 5 identical steps (2.5 x10 x 7.5 cm) is placed into a narrow corridor. A light bulb is placed above the apparatus to provide illumination and stimulate movement[26,31-35].

Method of Testing: Rodents are trained on how to climb the stairs by placing them on the apparatus with their head backing the stairs. Any animal that fails to move is tapped at the back, and the training continues for the period of 2 minutes[26,31,35]. After the training, the negative control group receives normal saline, positive control receives standard drug, and experimental groups receive graded doses of plant extract or fractions. Thirty minutes later each animal is placed into the staircase apparatus and allowed to explore it for 5 minutes. In some instance, food is placed at the top stairs as a reward to motivate the animal[26,31,35]. The parameters recorded include the number of upward steps climbed, the frequency of rearing and time took to reach the top over a period of 5 min for old apparatus and 3 minutes for the modified apparatus. The mouse was considered to have climbed a step if it places all its 4 paws on it. The apparatus is wiped with 70% ethyl ethanol to prevent olfactory cue[26,31,35].

Data Recording: Data can be recorded by playing the video or manually during the test to obtain the number of upward steps climbed, the frequency of rearing and time took to reach the top. The video can be played repeatedly to ensure accurate reading[26,31,35].

INTERPRETATION OF STAIRCASE METHOD RESULT

Number of Upward Steps Climbed: Increase in the number of steps climbed signifies an increase in motor coordination, on the other hand, decrease in the number of upward steps climbed implies poor motor coordination. A medicinal plant tested that decreases motor coordination posseses sedative property[26,31-35].

Frequency of Rearing: Decrease in frequency of rearing in an experimental animal group compared to the negative control without affecting the number of step climbing indicates anxiolytic property, while increase in the number of rearing in the test groups compared to negative control denotes anxiogenic activity of the medicinal plant or drug under investigation[26,31-35].

Time Taken to Reach the Top: Longer duration taken to reach the top of stairs indicates motor coordination deficit either due to the brain lesion or CNS-de-

pression. Increase in the time taken to reach the top in an experiment groups that were given plant extract reveal sedative property of the medicinal plant or drug under investigation[26,31-35].

LIGHT AND DARK EXPLORATION TEST

Light and dark exploration test was described by Crawley and Goodwin in 1980 as a simple behavior model to detect the anxiolytic action of medicinal plants, steroids and other compounds[50]. The technique is carried out to test for both anxiolytic and sedative action of compounds. In this test, a rodent is considered highly anxious if it spends more time in the dark compartment and lesser time in the lit compartment[19,36-39]. On the other hand, anxiolytic action is indicated by the increased time spent in the lit compartment and decreased time spent in the dark compartment[19,36-39].

Principle: This technique works based on the inherent rodents' aversion for brilliantly illuminated areas and natural tendency to explore their surroundings[36-38].

PARAMETERS RECORDED IN LIGHT AND DARK EXPLORATION TEST

Latency of Entry into the Dark Box: After placing the animal in this apparatus it may enter the dark box immediately or may hesitate to move. The time spent by the animal before making first entry into the dark/lit box is recorded as latency period[19,36-40].

Frequency of Entry into the Dark Box: Once the animal made first move into the dark box it should be tallied, up to the end of 5 minute-experiment. The total number of entries into the dark box will be recorded[19,36-40].

Time Spent in the Dark Box: Any time the rodent entered the dark box the duration of stay should be noted and recorded until the end of the experiment. The total time spent in the dark box is summed up as dark box duration[19,36-40].

Frequency of Entry into the Lit Box: The number of times the animal goes into the lit compartment is tallied until the end of 5 minutes. The total number of entries is then computed and recorded as frequency of lit box entry[19,36-40].

Time Spent in the lit Box: Immediately the rodent is placed inside the lit compartment, the time recorded before its making the first move is the latency period, recording of the time spent in the lit box begins after the rodent entered dark box for the first time and returned[19,36-40].

Frequency of Rearing: Rodents placed in a lit and dark box will be allowed to wander and observed closely, anytime the rodent raised its paws against the air will be recorded and finally summed as frequency of rearing[19,36-40].

LIGHT AND DARK EXPLORATION TEST EXPERIMENTATION

Apparatus: The light and dark box is a rectangular box made up of plywood (46 cm x 27 cm x 30 cm). It is divided into light and dark compartments. The two chambers have an opening in the middle measuring 7.5 cm x 7.5 cm. The floor of the apparatus is divided into nine squares of 9 cm x 9 cm each. The dark box is the smaller one which is 1/3 of the entire box with dimensions of 18 cm x 27 cm. The floor of the dark box is painted black[19,36,37,39]. The lit box is bigger and 2/3 of the entire box with the dimensions of 27 cm x 27 cm. The floor of the lit box is painted white. The entire top of the apparatus is covered by transparent glass to enable viewing inside. A video camera is placed 150 cm above the apparatus to record the animal behavior. The experiment is to be conducted in a small testing room with dimensions of 2 m x 5 m[19,36,37,39].

Method of Testing: The animals to be tested were brought to the testing room and allowed to acclimatize for 20 minutes. The animal is then placed individually inside the apparatus for 2 minutes to become familiar with the apparatus. Afterward, animals in the negative control group are given normal saline, those in positive control group receive standard drug

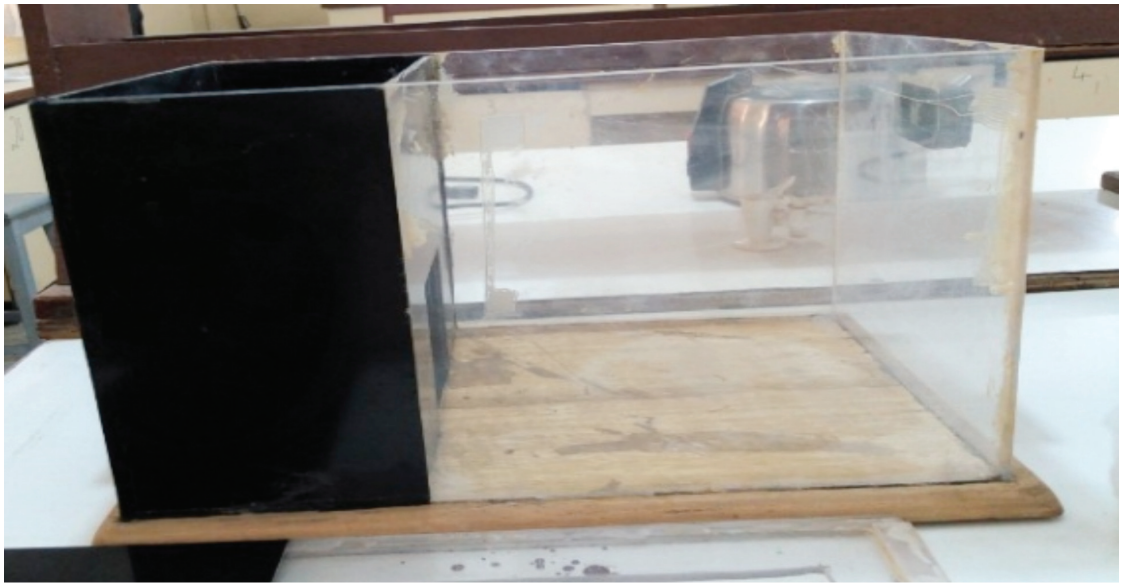

Plate 4. Picture of light and dark box apparatus taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

and those in the experimental groups receive graded doses of the extract or drug under test[19,36,37,39]. Thirty minutes later, the video camera is on otherwise the record is done manually; each animal is placed at the junction between the lit and the dark box with the head facing the lit compartment. The animal can explore the apparatus for 5 minutes. The inside of the apparatus is cleaned with 70% ethanol between each test to prevent olfactory cue. The observer is stationed at least 1m away from the apparatus in order not to interfere with animal activities[19,36,37,39].

Data Recording: Later the video is played, and the time taken by the animal to move into the dark box for the first time is recorded. Also, the number of times the animal entered the dark compartment and the lit compartment are recorded[19,36,37,39]. Finally, the total time spent by the animal in the dark box and the lit compartments are counted until the end of 5 minutes of recording. Notably, in the absence of video camera, same parameters can be recorded using a tally and a stopwatch[19,36,37,39].

INTERPRETATION OF LIGHT AND DARK EXPLORATION TEST

Latency of Entry into the Dark Box: Decrease in the time to enter the dark compartment is an indication of anxiety state, on the other hand, increase in the latency to move to the dark box is an indication of anxiolytic action of a medicinal plant or drug under test[19,36,38-40].

Frequency of Entries into the Dark Box: Decrease in the frequency of entries into the dark box by the experimental groups of animals compared to negative control reveal anxiolytic property. Nevertheless, increase in the rate of entry into the dark box unveils anxiogenic activity[19,36,38-40].

Time Spent in the Dark Box: An increase in the duration of stay in the dark box is an indication of anxiety state. However, decrease in the time spent in the dark compartment by the rodents that were given extract or any drug under investigation is an indication of anxiolytic property[19,36,38-40].

Frequency of Entries into the Lit Box: The more the experiment groups enter the lit box, the stronger the anxiolytic action of an extract or drug under investigation. However, a decrease in frequency of entry into the lit compartment signifies anxiety state[19,36,38-40].

Time Spent in the lit Box: An animal that is anxious tend to move away from the lit compartment. But animals that are given extract or drug under investigation may stay longer in a lit box if they have anxiolytic activity[19,36,38-40].

Frequency of Rearing: Rodents tend to rear less in an anxiety state but when given an extract or drug with anxiolytic property the frequency of rearings may increase significantly[19,36,38-40].

HOLE-BOARD TEST

Hole-Board experiment was introduced in 1962 to evaluate anxiety level and exploratory behavior of laboratory rodents[41]. It also determines both anxiolytic and sedative action of medicinal plant or any drug under investigation[41-42]. In this test, an increase in the frequency of head dipping by the experimental animal following the administration of a plant extract is an indication of an anxiolytic activity[42], while decrease in the frequency of head dipping by the test animals signifies sedative activity of the medicinal plant or drug under test[43-44].

Principle: Hole-board apparatus operates based on the natural tendency of rodents to show anxiety-like behavior when placed in an unfamiliar environment[41].

PARAMETERS RECORDED IN HOLE-BOARD TEST

Latency to First Head Dips: After placing the animal on the hole-board apparatus it may begin nose poking immediately or hesitate for sometime. The time taken to exhibit first nose poking is recorded as latency period[42-44].

Number of Head Dips: Rodents placed on the hole-board apparatus tends to explore the test environment and the holes around, the number of times the rodents poke their noses into the holes is recorded as number of head dips. The nose poking is said to occur whenever some rodents lowered both its two ears below the board surface[42-44].

Frequency of Rearing: Animal placed in this apparatus explore it from one corner to another. The frequency of rearing is counted anytime the animal raised its front paws against the air while standing on the hind limbs[42-44].

Plate 5. Picture of hole board apparatus taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

HOLE-BOARD TEST EXPERIMENTATION

Apparatus: The apparatus is made up of wooden board measuring 50 cm x 50 cm and 1.8 cm thick surrounded by transparent glass which is 5 cm high. Within the board, there are 16 holes each 1 cm in diameter, 2 cm deep and equally spaced. A video camera is stationed 2 m above the apparatus to record animal behavior[19,42-47].

Method of Testing: The animals to be tested are first brought to the laboratory 20 minutes before the experiment to acclimatize. In addition, each animal is placed on the hole-board apparatus for 2 minutes for habituation. Subsequently, the animals in the negative control group are given normal saline, while those in positive control group receive standard drug at a hypnotic dose and those in the experimental groups receive graded doses of the extract or drug under test[43-46]. Thirty minutes later, the camera is on otherwise data is recorded manually. Each animal is placed individually on the hole-board apparatus at one corner and allowed to explore the apparatus for 5 minutes. The latency to first head dips, number of head dips, and the frequency of rearing are observed and recorded. The apparatus is cleaned with 70% ethanol between testing[19,43-46].

Data Recording: The result of hole-board apparatus can be obtained by playing video records, otherwise it is done manually using tally and stopwatch. Recently, automated hole-board apparatus is introduced which records the behavior of animal such as latency to first head dips, number of head dips and frequency of rearing digitally[19,43-46].

INTERPRETATION OF HOLE-BOARD TEST

Latency to First Head Dips: Decrease in latency to first head dips is an indication of an anxiolytic property of the medicinal plant or drug under test. Nonetheless, increase in latency to first head dips signifies sedative property of the medicinal plant or drug under investigation[19,43-46].

Number of Head Dips: Increase in the number of head dips experimental animal groups compared to negative control is an indication of an anxiolytic action[42]. However, decrease in number of head dips in an experimental animal compared to negative control group signifies sedative property of the medicinal plant or drug under investigation[19,43-46].

Frequency of Rearing: In this test, increase in number of rearing in an animal groups implies anxiolytic action whereas decrease in frequency of rearing is an indication of sedative effect of the medicinal plant or drug under investigation[19,43-46].

BEAM WALKING ASSAY TEST

Beam walking assay is a behavioral study carried out to induce a minimal form of anxiety in rodents. It evaluates both anxiolytic and sedative property of a medicinal plant or any drug under investigation[26,31,46,48]. In addition, beam walking assay determines both motor coordination deficit and cognitive impairment in experimental animals[48-50].

Principle: This test operates based on rodents' natural aversion for height that is placing the apparatus 30 cm above the ground as well as the enthusiasm by the animal to reach the goal box[48-50].

PARAMETERS RECORDED IN BEAM WALKING ASSAY

Number of Foot Slips: When the animal is placed on the ruler with the head facing the goal box, it will begin to move until it reaches the goal box. Depending on how sedated they are after taking the plant extract, there may be foot slips which is tallied and recorded[26,31,46,48,51].

Number of Falls: Once the animals became heavily sedated, they may fall, apart from foot slips. The animal will be placed back on the ruler at the position where it fell for a maximum of 60 seconds. The number of times the animal fell will be tallied and recorded as frequency of falling[26,31,46,48,51].

Plate 6: Picture of beam walking assay test taken from pharmacology laboratory, Department of Pharmacology and Therapeutics, Bayero University, Kano.

Time to Reach the Goal Box: Animal placed on beam walking assay exhibits differences in the time taken to reach the goal box. The time taken by each animal to reach the goal box is recorded using stopwatch[26,31,46,48,51].

BEAM WALKING ASSAY EXPERIMENTATION

Apparatus: The beam walking assay apparatus consists of a ruler (80 cm long, 3 cm wide) placed in a platform and elevated 30 cm above the bench. A black box is stationed at the extreme end of the ruler[26,31,46,48,52]. A video camera is placed on a tripod stand 2 m meters away from the starting point. Otherwise, the record can be done manually using tally and stopwatch. Also, 60 watt- bright bulb is placed close to the starting point to serve as a stimulus to move. A nylon hammock is laid under the apparatus to serve as a cushion in case the animal fall[26,31,46,48,51].

Method of Testing: The animals to be tested are first trained to walk on a ruler placed on a platform, three attempts were made for each animal, the experiment is designed in such a way that the animal will be aware of a goal box to be reached. Subsequently, the animals in the negative control group receive normal saline; those in positive control group receive standard drug and those in the experimental groups receive graded doses of the extract or drug under test[26,31,46,48,52]. Thirty minutes later, a camera is on, and each animal is placed individually and allowed to walk towards the goal box. Each animal that fell is returned to the position they fell from up to a maximum of 60 seconds. Likewise, mice that turned back or fail to move were tapped at the back to stimulate movement. Sometimes food is placed during training as a motivation to reach the goal box very fast. The apparatus is cleaned with 70% ethanol between test[26,31,46,48,52].

Data Recording: The parameters evaluated are the time taken by the rodent to reach the goal box and the number of foot slips involving one or both hind limbs as well as number of falls. Recording can be done by the observer manually; otherwise, the video camera is used to record the whole event. The data is captured by playing the video to obtain the parameters needed[26,31,46,48,52].

INTERPRETATION OF BEAM WALKING ASSAY

Number of Foot Slips: Increase in foot slips in experimental groups compared to negative control is an indication of sedative property. Nevertheless, a decrease in the number of foot slips in experimental groups compared to negative control reveals anxiolytic action of the substance under investigation. Notably, anxiolytic property may occur at low dose while sedation at higher dose[26,31,46,48,51-52].

Table 1. Advantages and disadvantages of experimental models commonly employed in testing the anxiolytic activity of medicinal plants.

S/N	Test	Advantages	Disadvantages
1	Open Field Test	i. The advantage of an open field test is that it measures a wide range of parameters available[13]. ii. The temporal analysis allows the repeated measurement to be taken. This is important because animal behavior is a continuous process, not an event[13,17,22]. iii. The correlation that exists between parameters recorded can be established[13].	i. Open field test behavioral changes cannot be fully understood without taking cognizance into individual genetic variations[13].
2	Elevated Plus Maze	i. This test has face validity which is the ability to measure signs and symptoms of anxiety[22,30,53,54]. ii. Construct validity which is the ability to measure causes of anxiety[22,30,53-54]. iii. Predictive validity which refers to the ability to predict the activity of human drugs and their receptors[22,30,53-54].	i. This test is not an absolute determinant of animal behavior; other experimental models are needed to accurately determine the rodents' behavior[22].
3	Staircase Test Method	i. Parameters are easy to record manually[33-34]. ii. It also tests for the presence of a lesion in the brain. Thus, it is used for both experimental and diagnostic purpose[33-34]. iii. This test can detect anxiolytic activity at a much lower dose without producing sedation[33-34].	i. It measures only a few parameters[33,34]. ii. The apparatus is not automated as such accurate recording of behavioral parameters might pose a challenge[33,34].
4	Light and Dark Exploration Test	i. The apparatus was designed to enable animal behavior to be seen vividly[36,38,40]. ii. The experiment is easy to conduct and does not require training of the animal[36,38,40].	i. The test was initially designed only for male mice to avoid female reproductive hormones that are presumed to have anxiolytic property[36,38,40]. ii. The accuracy of data recorded defends on the researcher's familiarization with rodents and knowledge of animal psychology[36,38,40]. iii. It is difficult to extrapolate the result of the animal model into the human subject because human is stable to light stimulus[36,38,40].
5	Hole Board Test	i. It measures both anxiolytic and sedative property[47,55]. ii. The apparatus can be modified and joined with open filed to provide multiple functions[47,55]. iii. It determines both learning and memory[47,55].	i. The manual recording does not always produce an accurate number of head dips[47,55]. ii. Sometimes it is difficult to note whether partial or full nose poking has occurred[47,55].
6	Beam Walking Assay Test	i. Determines the extent of GABAA receptor occupancy more accurately than other experimental models[48-50]. ii. Uses ataxia to predict sedation in experimental animals[48-50]. iii. Rely upon sedation rather than motor coordination deficit to determine cognitive impartment[48-50].	i. After initial training, an animal that shows any sign of neurological deficit is not selected for the main experiment. Therefore, large numbers of animals were involved before getting an adequate sample[26,31,46].

Number of Falls: Although anxiety may cause the animal to fall from the platform, increase in the frequency of falls by an experimental animal that have taken extract signifies sedative activity. On the other hand, decrease in the number of falls implies anxiolytic property of the substance under investigation[26,31,46,48,51-52].

Time to Reach the Goal Box: Increase in the time taken by the rodents to reach the goal box is an indication of CNS-depression while decrease in time taken by the rodents to reach the goal box is an indication of anxiolytic property of the substance under investigation[26,31,46,48,51-52].

ADVANTAGES AND DISADVANTAGES OF EXPERIMENTAL MODELS

The advantages and disadvantages of experimental models discussed above are summarized in Table 1 below.

CONCLUSION

The behavioral study using animal models is a field of pharmacology that is attracting more researchers in Nigeria. Several pharmaceutical research centers and higher education institutions have contributed a lot to the anxiolytic testing of medicinal plants using animal models. Experimental models such as open field test, elevated plus maze, staircase test method, light, and dark box test, hole-board test and beam walking assay are the techniques commonly available and employed in the evaluation of anxiolytic activity of medicinal plants. Using these techniques, many medicinal plants with anxiolytic property claims have been proven and validated. Interestingly, the majority of these apparatus can be improvised in an environment where they are not available. The overall procedures are easy to carry out and yield a reproducible result. Behavioral studies can advance further in Nigeria by the introduction of automated experimental techniques and making them available to young researchers.

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