REVIEW ARTICLE

Pharmacological models to appraisement of antianxiety activity in experimental animals

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Abstract

The disease related to brain is very challenging task in the area of neuroscience research. Anxiety is one of the central nervous system disorders in which human feel excessive and unusual fear. Several antianxiety medicines available in market to treat and suppress this problem, but there is lack of evaluation methods available for them. The present review contains all the models such as plus maze apparatus, zero maze apparatus, forced swim apparatus, and open field methods, which are available for pharmacological evaluation of antianxiety. Accuracy can be achieved using these models in a comparative way and one has not to depend on any one model. Several modifications are also possible with these models to achieve accurate and precise result. Working principle of models is enlisted in this review to make them better understandable.

Key words: Actophotometer, anxiety, cognition, Hole board, plus maze, Rotarod apparatus

INTRODUCTION

Science has answered many questions around the world, but still there are several questions, which are not yet answered by science.[1] In field of pharmacological science, it is yet difficult to answer that question which is related to our brain and human psychology.[2,3] Brain is very complex structure that cannot be fully understood as such, for that, we have to continuously develop and modify our methodology. To answer questions related to brain and other nervous system pharmacologist working hard by involving new and innovative experimental models for the evaluation of disease such as anxiety, depression, sedation, excitement, Parkinson's, and many more.[4] In this review, different models are listed which are used in the evaluation of antianxiety activity in rat and mice.[5]

Health is defined as a state of complete physical, mental, and social well-being.[6,7] Anxiety is condition, which cause unwanted fear in individuals' mind. It may be fear of losing someone or getting die, sometime a small thing could be a reason of anxiety in population. According to the WHO study, there are about 10% of people in the world are suffering from acute or chronic anxiety.[8] Anxiety is a universal human experience, it is not a disease, and it is associated with several psychiatric disorders. Exact reason of anxiety is yet to be found, but it is believed that it is due to improper propagation of chloride channel and this is cured using different medicine of benzodiazepine categories.[9]

ANXIETY

In one word, we can say that anxiety is a feeling of fear. The object of fear may be true or that may be just an imagination, often general causes of anxiety are - tension of examination, arrangement of money for survival of family, the incident that had already happened, fear to stand in front of crowd, and

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speaking among them.[7,10-15] Worries, doubt, and fear are a normal part of life. It is natural to be anxious about our upcoming test or to worry about our financial condition. The difference between normal worrying and generalized anxiety disorder is that generalized anxiety is excessive, intrusive, persistent, and debilitating.[16] Anxiety disorder is becoming one of the common problems in our society it affects about 40 million adult of age group 18 years and older.[17] In animal model study, it has been seen that height, hunger, loneliness, disease, thrust, threatening condition, violence, pain, darkness, and different stimulus such as current, heat, cold, pinch, and self-image are used for the development of anxiety.[18] The limbic system of brain located in the most primitive part of the cortex and in the seat of the emotions.[19-21] The reticular formation of the brain is involved in anxiety states. Thus, different models that have been made by researchers are based on different principle and methodology so we cannot judge the efficacy of any reference or experimental drug. One common principle among all of these models is that they are based on the behavior of experimental animal.[22]

Symptoms of Anxiety

The major symptoms of anxiety are listed below:

1. Fear, nervousness, dizziness, swatting, palpitation, and uncontrolled urination.
2. Headache, sleeplessness, bradycardia, hypertension, isolation feelings, a feeling of imminent danger, or doom.
3. The need to escape, heart palpitations, trembling, shortness of breath or a feeling of choking, chest pain or discomfort, nausea, or abdominal discomfort.

Types of Anxiety

Generalized anxiety or acute anxiety

Acute anxiety is a simple anxiety, which can be easily diagnosed and treated by some changes in behavior. The causes of acute anxiety include the arrangement of common household goods such as money and food and to have a better and luxurious life.[23] These are not fatal. Fear is specific type of emotional state and this may be classified in different types such as fear of failure, fear of rejection, fear of death, fear of isolation, and fear of the unknown. Fear of failure is a condition in which we get worried about the failure of any specific task, which we are going to perform [Table 1]. In actual case, it is the expectation by others on us. Normal worry and generalized anxiety can be differentiating by the following symptoms.[24]

Panic or chronic anxiety

Chronic anxiety is a result of untreated acute anxiety, if acute anxiety is not treated with medicine or other method, then it will convert into chronic anxiety and it may be dangerous for patient. Chronic anxiety can be treated by medication only and other methods such as love, support, and arrangement of financial help can support the patient to recover the patient in less time [Table 1]. The role of histamine has also been reported in anxiety disorder as if histamine content is low, then extent of anxiety will be high.[26]

Table 1: Comparison between normal worry and generalized anxiety

Normal worry	Generalized anxiety
Worry does not affect daily activity	It affects our day-to-day activity
Normal worry can be control by our self	The generalized anxiety cannot be control without the use of medication[25]
Although worry is unpleasant, it does not cause significant distress	Cause significant distress
This worry is limited to a small number of reason or factor	The causes of generalized anxiety may be more than one or two reason
The duration of worry can last up to maximum of 3–4 days	If untreated, then it may convert into chronic anxiety

Post-traumatic anxiety

Post-traumatic anxiety means the fear of any incident, which had already been happened. The memory always flash and person get anxious with that. A traumatic event must be faces or seen by the patient in his life like death of any close one in accident, injury, physical integrity of himself or others, violent crime, military attack, and being kidnap are the common example of post-traumatic anxiety. Sexual harassment in childhood is one of the common post-traumatic stress disorders, it is very often that we neither say anything nor inform anyone about this incident and it takes a corner in our mind and whenever we become alone it forcefully come in front of us. Post-traumatic anxiety is treated with lots of love and affection.[27]

Obsessive-compulsive disorder (OCD)

It is also known as OCD. Obsession and compulsions are the important component of OCD, and the patient who is suspected, as an OCD patient must show any of these criteria. Ideas which are continuously running in mind thoughts which we think, our imagination and other things which are inappropriate and which cause stress comes under obsession and compulsions can be defined as the activity, behaviors, and many thoughts which we do to get relief from anxiety is known as compulsion. Simple most example of this category is to wash the hand each time when we touch the handle of door.[27]

ANTIANXIETY DRUGS AND THEIR MECHANISM OF ACTION

Drugs used to treat anxiety by maintaining the normal calm level of body and brain is known as antianxiety drugs.

Antianxiety agent was formerly called minor tranquilizer and known as anxiolytics tensiolytics.[28] They are chemical agent, which are used to control the effect of stress, discomfort, fearful anticipation, and dysphonia in patients with neuroses and mild depressive state. These drugs are used in special condition only because they have tendency to cause addiction and habituation, the patient may become addicted from their regular dose. Antianxiety drugs are classified into different categories such as benzodiazepine, barbiturates, benzodiazepine antagonist, and other hypnotic agents.[14] Several drugs such as alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, lorazepam, flumazenil, antidepressant, amobarbital, pentobarbital, phenobarbital, antihistaminic, chlorhydrate, and eszopiclone are the few examples of antianxiety drugs.[7,8,29,30]

Anti-anxiety drugs acts on GABA receptor and they open the chloride channel and extend the penetration of chloride channel through it, chloride channel are responsible for the negative charge inside cell, after some time negativity got balanced due to presence of potassium ion, and thus the normal physiology of body maintain by the continuous polarization and depolarization process.[12] However, when GABA channels are open by antianxiety drug, the penetration of chloride channel increases inside cell and when negativity increases it also increases polarization, but this generated polarization is comparatively longer than normal polarization, thus this is also called hyperpolarization [Figure 1]. Hyperpolarized condition delays the depolarization state and these moves the postsynaptic potential away from action threshold and inhibit the action potential. The benzodiazepine drugs show some other activity other than antianxiety, these activities correlate them self in then evaluation. Other activities include - sedation and hypnosis, anterograde amnesia, anticonvulsant activity, and muscle relaxant activity.

MODELS USED TO EVALUATE ANTIANXIETY ACTIVITY

Models used for the evaluation of antianxiety activity are based on the changes in mental behavior of experimental animal before and after the experiment.[27,31,32] Different models are used for the stimulation of anxiety and then administration of drug shows their therapeutic effect by calming and reducing anxiety. Animal models of emotional disorders attempt to reproduce the behavior and nature of human patient disorder in different laboratory animal correlating the physiological and behavior changes associated with specific emotional state. It is believed that the animal model, which we use to reproduce the behavior of human, will be same, but it is not always possible to get the satisfactory result, due to this disadvantage one must not rely on any one model. In most of animal model, the attempt is made that benzodiazepine will restore the normal physiology of animal behavior which has been altered by different anxious stimuli.[33] Behavioral model of anxiety is based on observation of reaction of experimental animal used for test, in this the change in their normal attitude in the presence of stimuli and recovery to their normal state after giving antianxiety drug. Models that are already available can be used for the evaluation and different other models, which are similar in principle, can be employed.

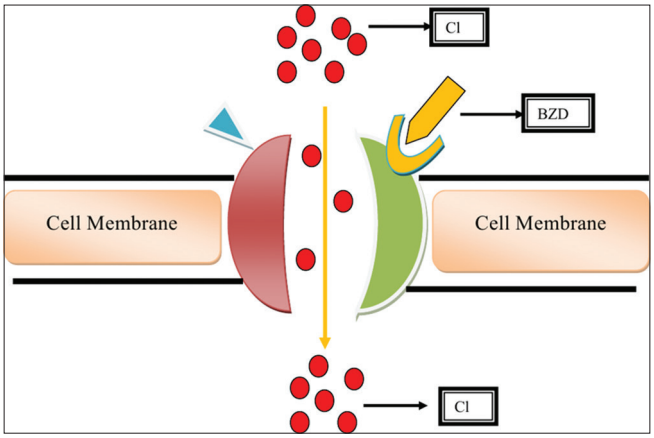

Figure 1: Mechanism of action of antianxiety drugs

Behavioral models are basically divided into two types - (1) exterioceptive means those originating outside the body and (2) interoceptive which originating within the body, they also known as unconditional model and conditional model, respectively.

Exterioceptive or Unconditional or Ethological Model

All models come in this category exist same principle that subjects are exposed to aversive stimuli which result in readily observed changes in the behavior of the subject. This anxious behavior can be restoring by the administration of antianxiety drugs. In this procedure, no training is required and collection of data is rapid [Table 2]. When the subject is placed in the uncomfortable situation, its learning capability is suppressed and this is a central feature of human anxiety.[34]

Interoceptive or Conditional Models

In a model, several stimuli are used to generate the anxiety. Different examples are available to justify this principle like application of electrical stimulation at any point of brain, administration of anxiogenic drugs is used to create anxiety. Interoceptive models are much important in use because they are analogs to human anxiety as it is generated inside the body [Table 2]. For the assessment of antianxiety activity of any drugs in human body, we must use this model than the unconditional model. In human anxiety is produced using anxiogenic drugs such as amphetamine, cocaine, bemegride, pentylenetetrazole, strychnine, caffeine, and benzodiazepine-binding site antagonist can be used for this purpose.

Elevated Plus Maze Apparatus

This apparatus has four arms, each in every side, two sides are open and two sides are closed, open side means the arms

are not having any wall or border at their corner and they usually allow light from surrounding, and the closed sides are just opposite of this property [Figure 2]. Elevated means that apparatus (Plus Maze) is kept at fix height from base.[35-37] Rodents have tendency to live in dark places and they usually avoid the light and bright places, they also have anxiety with height.[31,38-41]

1. When we keep rat/mice in the center of apparatus facing toward open field, first, they took time to familiarize themselves and then they used to move across all the four arms.
2. After traveling to all corners, they came back to the closed side as this side has covering and protective walls and these sides are dark too.
3. Different parameters are studied like in 5 min time how much time they enter in open and closed side and how much time they spent in each side.

Construction detail of plus maze apparatus:

a. 32 cm length of open arm means 16 + 16 cm one side and 12 + 12 cm on closed side and 5 cm width of each arm. This measurement is for mice.
b. 100 cm length of open arm means 50 + 50 cm one side and 40 + 40 cm on closed side and 10 cm width of each arm. This measurement is for mice. The elevation is about 50 cm from bottom.

Table 2: Different animal models of anxiety evaluation

Animal models of anxiety evaluation	
Unconditional test models	**Conditional test models**
Elevated plus maze apparatus	Geller–Seifter test
Open maze apparatus	Vogel conflict test
Elevated Y maze apparatus	Emotional conditioning responses
Elevated zero maze	Ultrasonic conditioning vocalization
Elevated T maze	Fear-potentiated startle
Rotarod apparatus	Place aversion test
Dark-light model	
Mirror chamber	
Isolation from group	
Tail suspension method	
Forced swim test	
The Hole-board test	

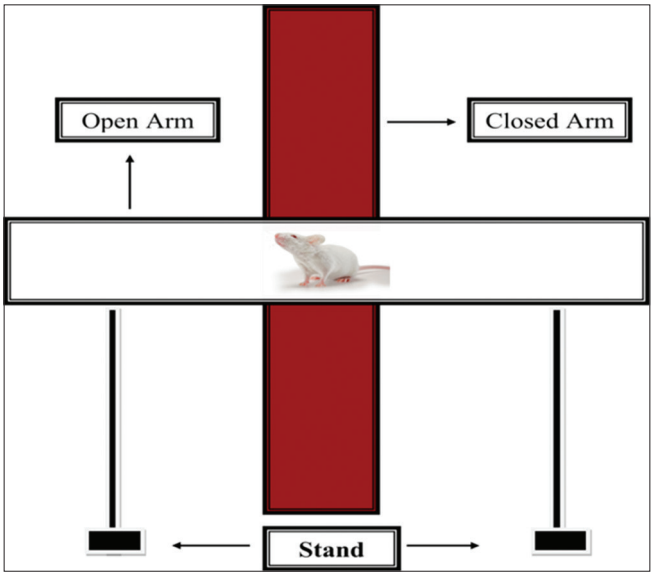

Figure 2: Plus Maze apparatus

The Elevated Zero Maze (EZM) Apparatus

As name indicates, it is an apparatus, which has the shape of zero, and it is elevated to the height from bottom or ground. Actually, it is the modification of elevated plus maze apparatus that incorporates the both traditional and novel method of evaluation. The EZM is a circular runway elevated from the floor that alternates open, brightly light area with enclosed, dark path.[32,36]

Elevated T Maze

Graeffy introduced the elevated T maze. It is based on the principle of plus maze apparatus and consists of three arms, which represent its T-like structure, one enclosed by lateral wall standing perpendicular to two opposite open arms of equal dimension. The whole apparatus is elevated from floor.[32] This model allows the measurement of two different behaviors in same animal at same time. On 1 day before the test, animals are exposed to the apparatus by placing them in each of the arm so that they may familiar with apparatus and will not take a time to respond while actual activity will be performed.[15,36,42]

Open Field Test

Broadhurst first described open field test in 1969, this behavior test is based on the induction of different mixture of anxiolytic stimulus such as freezing animal by high pitch sound and light and flashing accessories.[32,41] It is consist of circular area of 27 cm height and 84 cm in diameter with 25 houses equipped with light and sound sources. Animal is then placed one by one in that circular path at the center of that [Figure 3]. When animal moves from one place to another one score is recorded, similarly when the animal stands on its hind limbs with or without the support of wall one rearing (complex) score is recorded.[28]

Rotarod Apparatus

Rotarod apparatus is used to evaluate the muscle relaxant property of any drug, but it can also be used in the evaluation

of antianxiety activity.[39,40] Drug which causes depression of motor function, leading to relaxation of voluntary muscle is known as muscle relaxant. Common sign of antianxiety drugs is muscle relaxation. Whenever, we introduced any antianxiety drug in any experimental animal, then the drug produces relaxation of muscle. These can be correlating with its efficacy. Rotarod apparatus can be easily assembled and can be made at classroom or experimental room [Figure 4].

1. This instrument consists of a rod, which is connected to a rotating belt at specific RPM (about 15–20) at one end, the rate of rotation of the rod should be adjusted to that speed that a normal mouse can stay on plane or the rod for an appreciable period, generally, about 3–5 min of time.
2. The platform of apparatus is fitted with an auto cut device, which gets stop when rat/mice fall down; otherwise, it continues its countdown.
3. When mice are placed on rotating rod mice try to hold it as tightly as possible, and this causes anxiety, after some time, it will fall down and this time will be noted.
4. After administration of antianxiety drug, this response time will be shorter than before as the anxiety will be reduced by antianxiety drug and relaxation of muscle is another sign of antianxiety drugs.

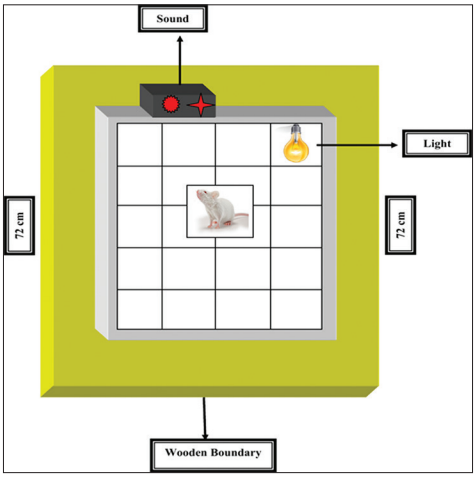

Figure 3: Open field model

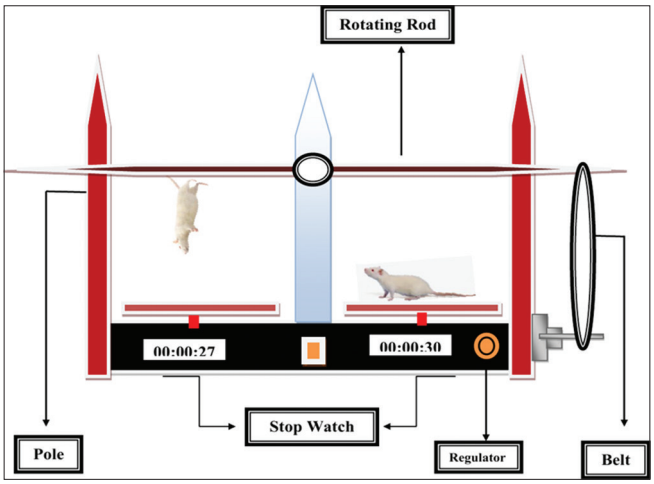

Figure 4: Rotarod apparatus

Specification:

1. Rotation should be about 20–25 per min
2. The height of rod from bottom is about 20 cm.

Dark-light Model

Grawley introduced dark-light model or apparatus and Godwin in 1980 and later Costal in 1989 validated the black and white test box model by variation of illumination within the test box and the use of different strain of mice. It is very simple in construction, it is made up of a wooden box having two chambers one bigger and one smaller which were separated by a thin wall, but a tiny gate is there to allow animal (rat) to pass between these two chambers [Figure 5]. Light-dark chamber was given its name because the longer chamber is painted in white (light) color, and smaller chamber is painted in black (dark) color.[43]

1. We may place light source in light chamber side.
2. Upper side of apparatus is covered with a wooden plate. Now, place a mice/rat in light chamber and allow him to travel between these two sides.
3. The total time spent in each side is counted for a specific time (usually 5–10 min) or total entry in each side is also counted.[36,48]
4. This experiment is performed before and after the drug administration.
5. The exact measuring can be done by scaling at the bottom of light and dark chamber so it will be easy to detect the distance traveled.
6. At the last of experiment, total movement of rat in light chamber and total time spent in chamber is recorded. The same procedure is applied for dark chamber also.

Specification:

1. 45 cm × 27 cm × 27 cm wooden or plastic chamber[27]
2. Base is divided into 9 cm^2
3. Separated by a thin wall just between boxes
4. One small passing door is made in the wall section
5. One side (two-fifth) is polished with black paint and one side (bigger) is painted by white paint
6. A light source (red light) is located at 17 cm above the box
7. Total entry and total time spent in each side are recorded.

Mirror Chamber

Mirror chamber is an apparatus, which consists of several mirrors in each side of a wooden box to create multiple images. The mirror is placed in a side creating a passage space for experimental animal. The surrounding is painted in dark brown color and the passage of about 5 cm is left around the wooden box.[31,41] When rat or mice are left in box, they travel through the passage one by one and we note the latency of rat to enter the chamber that means the time which rat required to enter in mirror box and number of entries in 5-min time and the second entry in the chamber during 5 min test periods. When they enter inside the mirror box where they feel the

presence of several mice inside and they become anxious, they show different activity as if they touch their image and try to bite them, they try to touch and many activities that are more similar. After the administrations of antianxiety drug, their behavior changes, they become calm and less interactive.[36]

Specification:

1. Container or wooden box is about 40 cm × 40 cm × 30.5 cm length
2. Squire wooden box height is 30.5 cm
3. Length of wooden box is about 40cm
4. Height of mirror cube is 30 cm in all dimensions painted dark brown in their back
5. Three mirrored side panes, a top pane, and floor pane faced the interior of the cube
6. The mirrored cube is placed on the box leaving a 5 cm corridor.

Isolation from Group

In this method, a number of rat/mice are kept in group and then allow them to acclimatize in that atmosphere for about 3–4 days, then separate one to two of animal from society one by one and keep them in a single cage and absorb their behavior, how they are behaving in loneliness. There is another modification of this procedure, instated of observing behavior of isolated animal the behavior of left animal can also be simultaneously studied. In this procedure, all animals of a group are separated one by one and the last animal which is remaining shows a different activity than previous animal, there is increase in rectal temperature in remaining rat. This separation creates or initiates the anxiety in their mind and they start behaving accordingly. Now, the standard and experimental drug can be simultaneously injected in rats which have been separated and then start observing them again, different response will be obtained.[36,43]

Tail Suspension Method

Tail suspension method is one of the behavior activity models, which is used to perform antianxiety and antidepressant activity in mice. In this method, the experimental mice are suspended by its tail to the specific height and allow it to behave accordingly. At the starting of few minutes, it will try to make itself free, then this tendency will reduce slowly-slowly as it becomes anxious and depressed [Figure 6]. After some time of drug injection, it will again start to make itself free. This response is noted and then compare with the standard drug.[31]

The Hole-Board Test (Head-Dipping Apparatus)

The hole-board test apparatus is consist of a square wooden structure in which number of holes are present at its floor. Animals are subjected to the hole-board test for 5 min. The rodent can expose by poking their hands. This testing apparatus is now known as head-dipping apparatus which has been validated as a measure of exploratory activity and anxiety [Figure 7]. The number of head deep is assumed to be inversely proportional to the anxiety state; it means if the experimental rat deeps its head more times in the hole presents the extent and strength of anxiety will be less and *vice versa*.[35,36]

Foot Shock-induced Aggression

Neurological and pharmacological evidence suggest that involvement of GABAergic mechanism in the control of aggressive behavior in animals. Benzodiazepines produce

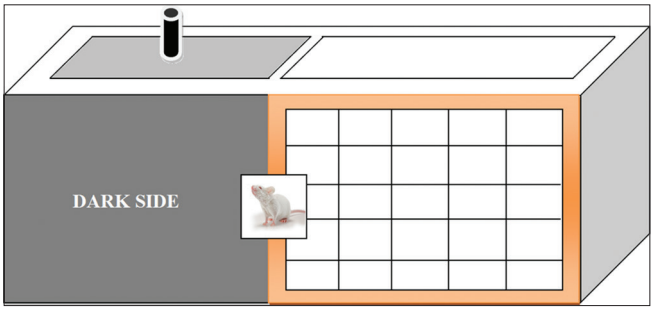

Figure 5: Dark-light model for anxiety

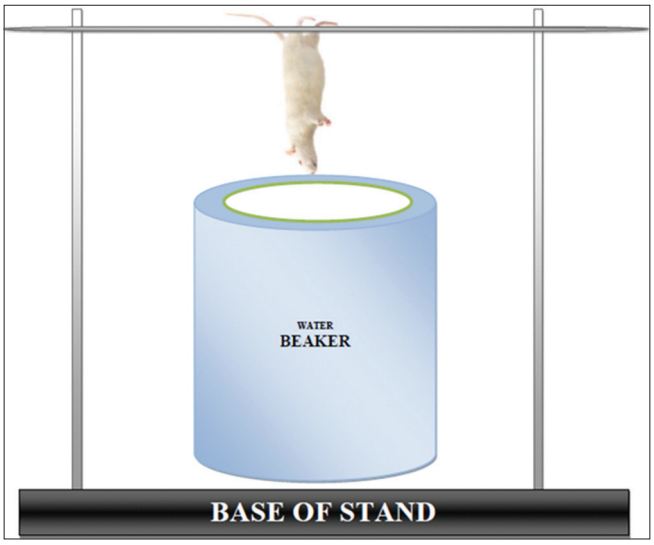

Figure 6: Tail suspension method

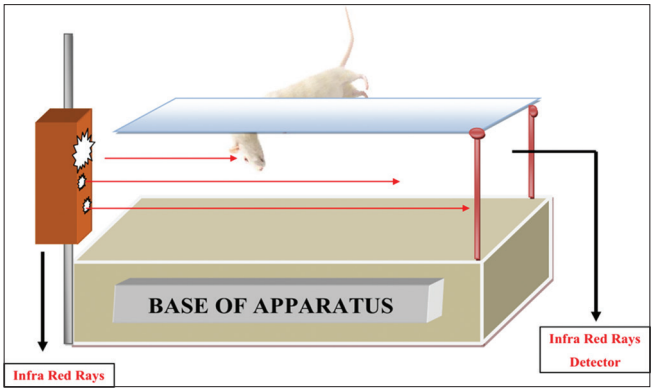

Figure 7: Hole-board test apparatus

anxiolytic effects by binding to a specific high-affinity site on GABA-A receptor. Aggressive behavior in mice has been induced in mice by allowing them into contact with the area which generates the threshold potential of about 60 V for a time of nearly 1 min in aggresometer. When animal in the experiment feel the shock generated by current, it tries to stand on its own and comes closer to each other and start biting equally.[27,47] Their behavior can be observed and calculated using the following formula:

1. Touching each other - 1 point
2. Single fight - 2 points
3. Biting and fighting - 3 points
4. Bleeding and fighting - 4 points
5. Control reading is taken in 24 h before the experiment.

Radial Water Maze

This apparatus is used for both learning memory observation and evaluation of anti-anxiety efficacy. It is very much similar to radial arm maze, but little modification is done in this apparatus.

1. It consists of a circular pool of 120 cm in diameter and 60 cm in height
2. Filled with 30 cm deep with water at 25°C and made opaque by adding milk or a non-toxic white color
3. Eight 12 cm wide channels formed by a 40 cm × 40 cm plastic wall project readily from the central area that is 30 cm in diameter
4. A pneumatic device can move a circular platform in the middle of the central area vertically between the upper position 1 cm above the water surface and lower position at bottom of the pool
5. The animal undergoes one trail per day. At the beginning of each trail, the rat is placed on the central platform of the maze
6. After 10 s, the platform lowered and the rat forced to swim until it reaches a bench at the far end of one of eight arms and climbs on it.

Actophotometer

It is clear indication that the drugs, which affect the locomotor activity of experimental animal, will surely affect its anxiety level and for validation purpose. Actophotometer used for the measurement of locomotor activity. It is a box of square size equipped with photoelectric cells, which are connected in circuits with a counter. Its number may be different in different instruments. Photoelectric cells are arranged in one side of box and on the opposite side of cell same number of light source are installed. When the beam of light source is allowed to travel through box it reaches the photoelectric cell and thus circuit become complete and counter does not count the reading, but when there is any interruption in light beam and light will not fall on the photoelectric cell there will be interrupted circuit and counter will count the reading [Figure 8].[40] Different antianxiety drugs such as diazepam, clonazepam, and alprazolam are effectively evaluated by this instrument.[44-46]

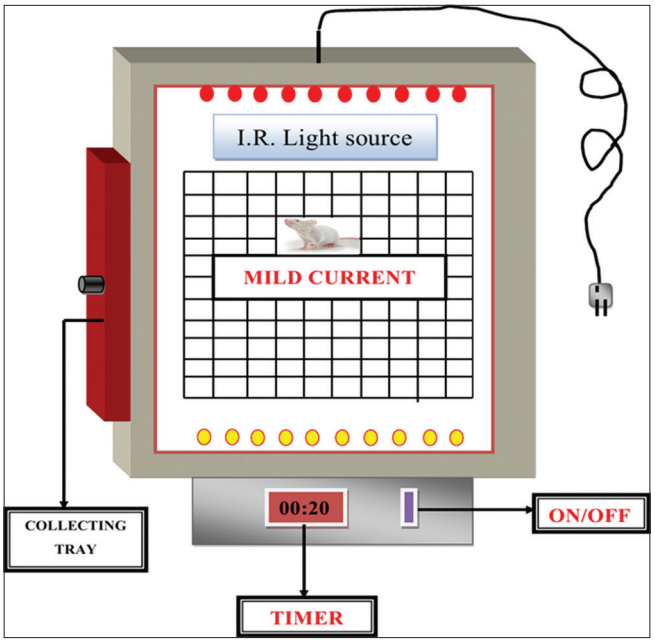

Figure 8: Actophotometer apparatus

CONCLUSION

We came to the final of our study that as in current scenario almost every two of five adults are facing the problem of anxiety and other social-related mental retardations. They have unnecessary and unwanted fear for the day-to-day life like for office performance, although this is acute type of anxiety, there is strong possibility for conversion into chronic one. Neurological disease, especially anxiety, is very vast area to understand. Many pharmacological models are there for the evaluation of efficacy of antianxiety drugs, but they do not provide satisfactory results, thus for obtaining accurate and consistent result, we must know the principles of all available models so that they may interpolate all together. All neurological problems are showing different response to their used screening models. Depending on the type of anxiety and its severity, one should choose the evaluation parameter carefully. Plus maze apparatus, dark light model, and mirror chamber are commonly used, but actophotometer, radial maze apparatus, isolation anxiety, foot shock, and radial water apparatus are now in use. These studies are very useful for the researcher, physicians, and other medical professionals related to this field.

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