NEUROPHARMACOLOGICAL SCREENING TECHNIQUES FOR PHARMACEUTICALS: A REVIEW

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Summary

This review presents various models to screen various traditional drugs and modern drugs for neurological effects. This brings a compilation make easy to go through the various models at same time and make it easy to screen the drugs. It reveals principle, apparatus and procedure of these models. This screening model can be use to study of memory enhancing, Anxiolytic, Antidepressant and anticonvulsant activity.

Keyword: Scopolamine; Elevated plus maze; Shuttle Box Avoidance; Muricide behaviour; Pentylenetetrazole (PTZ); Electro Shock (MES);

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Introduction

The Central nervous system (CNS) comprising of the brain and spinal cord process information with the help of chemical messenger viz. neurotransmitter, neuromodulators, neuroregulators, neuromediators and neurotropic factor which act via specific mechanism to mediate neurotransmission, neurotransmitter viz., nor adrenaline, adrenaline, dopamine, Gamma Amino Butyric Acid (GABA), glutamate, acetylcholine, 5-hydroxytryptamine (5-HT), peptides viz. endorphins, seretonin, glycogen and vasoactive intestinal polypeptides(VIP) etc and neuromodulator viz. prostaglandins (PGs), purines and neuropeptides interact with their recognition sites i.e. receptor and regulate the function of CNS (1).
According to the world health report (WHO 2001) approximately 450million people suffer from a metal or behavioral disorder, yet only a small minority of them receive even the most basic treatment this amount to 12.3% of the global burden of disease and will rise to 15% by 2020 (2).
Drug acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from the vast array of material medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering (3).

In the search of new therapeutic product for the treatment of neurological disorder medicinal plant research worldwide has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (2).
Many classical animal models of preliminary pharmacological tests of activities on CNS which provide information about action upon psychomotor performance, motor behaviour and neurotoxicity .The depression activity gives an indication of the level of the excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS (4).
This review presents various models to screen various traditional drugs and modern drugs for neurological effects. This brings a compilation make easy to go through the various models at same time and make it easy to screen the drugs. It reveals principle, apparatus and procedure of these models.

Neuropharmacological Screening Techniques

1. Memory Enhancing Activity

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning and memory processes (5). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (6) and human beings (7).Epidemiological studies of Indian population reveal that dementia is largely a hidden problem (8). Prevalence rates for dementia increase exponentially with advancing age (9&10). Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients. Various laboratory models for testing learning and memory are

(i) Scopolamine induced amnesia (Interoceptive Behaviour Model).
(ii) Diazepam induced amnesia (Interoceptive Behaviour Model).
(iii) Elevated plus maze (Exteroceptive Behaviour Model).
(iv) Shuttle box avoidance (Two-way shuttle box)
(v) Passive avoidance paradigm (Exteroceptive behavior models)

1.1. Scopolamine-Induced Amnesia in Mice:

Principle

The administration of the antimuscarinic agent scopolamine to young human volunteers produces transientmemory deficits (11). Analogously,scopolamine has been shown to impair memoryretention when given to mice shortly before training in a dark avoidance task (12,13,&14). The ability of a range of different cholinergic agonist drugs to reverse the amnesic effects of scopolamine is now well documented in animals and human volunteers. However, the neuropathology of dementia of the Alzheimer type is not confined to the cholinergic system (15)**.**

Procedure

The administration of the antimuscarinic agent scopolamine to young human volunteers produces transient memory deficits. Analogously, scopolamine has been shown to impair memory retention when given to mice shortly before training in a dark avoidance task.

The scopolamine test is performed in groups of 10 male NMRI mice weighing 26-32g in a one-trial, passive avoidance paradigm. Five minute after i.p. Administration of 3 mg/kg scopolamine hydrobromide, each mouse is individually placed in the bright part of a two-chambered (bright and dark) apparatus for training.
After a brief orientation period, the mouse enters the second darker chamber. Once inside the second chamber door is closed which prevents the mouse from escaping, and a 1 mA, 1 -see foot shock is applied through the grid floor. The mouse is then returned to the home cage. Twenty-four hours later, testing is performed by placing the animal again in the bright chamber. Latency in entering the second darker chamber. The latency in entering the second darker chamber within a 5 min. test session is measured electronically. Whereas untreated control animals enter the darker chamber in the second trial with latency about of 250 see, treatment with scopolamine reduces the latency to 50 sec. The test compounds are administered 90 min before training. A prolonged latency indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber.
Using various doses latencies after treatment with test compounds are expressed as percentage of latencies in mice treated with scopolamine only. In some cases straight doses-response curves can be established whereas with other drugs inverse U-shaped dose-responses are observed (14).

1.2. Elevated Plus-Maze (Exteroceptive Behaviour Model)

Principle
Out of many possibilities to modify maze tests e.g. water maze (16), the Y-mace, the radial maze (17), and the elevated plus maze (18 & 19) have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect.
Procedure
Elevated plus maze served to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory is followed as per parameters described by the investigators working in the area of neuropsychopharmacology
The apparatus consisted of two open arms (16cm x 5cm) and two closed arms (16cm x 5cm x12cm). The arms extended from a central plat from (5cm x5cm) and the maze is elevated to a height of 25 cm of the floor. On the first day, such mouse is placed at the end of an open arm, facing way from the central platform. Transfer latency (TL) is taken by mouse with all its four legs to move into one of enclosed arms .TL is recorded on the first day. If the animal did not entered arms within 90 sec. It is gently pushed into one of two enclosed arms and TL is recorded at 90 s. then three consecutive mice are allowed to explore the maze for another 10s and then returned to its home cage. Retention of this learned –task is examined 24 H after the first day trial. Another laboratory model viz. passive avoidance apparatus was employed to substantiate the findings and overcome the limitations of the elevated plus-maze.

1.3. Shuttle Box Avoidance (Two-Way Shuttle Box)

Principle
Compared to runway avoidance, shuttle box avoidance (two-way-shuttle-box) is a more difficult task. Since the animal is not handled between trials, the shuttle box can be easily automated (20).

Procedure

Compared to runway avoidance, shuttle box avoidance (two-way- shuttle-box) is a more difficult task. Since the animal is not handled between trials, the shuttle box can be easily automated.

Rats of both sex are used and maintained under standard conditions. The apparatus used consist of a rectangular box 50 x15 cm^2 with 40 cm high metal walls, and an electrifiable grid floor. The box is divided by a wall with a manually or solenoid operated guillotine door (10 x 10 cm^2) into two 25 x 15 cm^2 compartments. Each compartments can be illuminated by a 20 W bulb mounted in the hinged Plexiglas lids. A fixed resistance shock source with an automatic switch (0.5 sec on 1.5 sec off) is used. Simple programming equipment provides for automatic delivery of the command stimulus (CS) and the unconditioned stimulus (US). The apparatus is placed in a dimly lit room with a masking noise background (whit noise) of 60 dB. The animal is allowed to explore the apparatus for 5 minutes with the connecting door open and the compartment lights switched off. The guillotine door is then closed. 20 sec the light is switched on in the compartments containing the animal and the door is opened. A tone (CS) is presented and 5 sec later the floor shock is applied in this illuminated compartment and continued until the animal escapes to the dark side of the compartment; the connecting door is close and the shock discontinued. After in variable inter trial (ITI; 30-90Sec) the light is switched on in previous dark compartment, the door is opened and animal is required to cross to another side. The training is continued until animal reaches critical of 9 avoidances in 10 consecutive trials. Retention is tested at different interval after the original training by the retaining the animal to same criterion again.

The animal need to reach the safe on both days is measured. In addition the number error (not reaching the safe area) is recorded.

The task is rather difficult due to lack of permanent safe area 'lack of simple instrumental response, presences of variable aversive gradient and increased weight of emotion factor.

1.3. Passive Avoidance Paradigm (Exteroceptive Behavior Model)

Principle:

One of the most common animal tests in memory research is the inhibition to imitate activities or learned habits. The term "passive avoidance" is usually employed to describe experiments in which the animal learns to avoid a noxious event by suppressing a particular behaviour.

Procedure:

Passive avoidance behaviour based on the negative reinforcement used to examine the long term memory (21 & 22). The apparatus considered of a box (27cm x 27cm x 27cm) having three vales of wood and one wall of Plexiglas, featuring a grid floor (3mm stainless steel rods set 8mm apart), with a wood platform(10cm x 7cm x 1.7cm), in center of grid floor. The box is illuminated with 15 W bulbs during experimental period. Electric shock (15V AC) is delivered to the grid floor. Training is carried out in two similar sessions. Each mouse gently placed on wood platform set in center grid floor. When the mouse stepped down place all its paws on the grid floor, shock is delivered to 15 sec and step- down latency (SDL) is recorded. SDL is defined as time taken by mouse to sleep down from wood platform to grid floor all its paws on grid floor. Animal showing the SDL in rang (2-15 sec) during first test are used for the second session and the retention test. The second session carried out at 90 min after the first test. When animal stepped down for period of 60sec retention is tested after 24 h in the similar manner, except electric shock is not applied to grid floor. Each mouse again place on the platform and the SDL is recorded, with the upper cut of time of 300sec.

Anxiolytic Activity

Principle

The animal model is considered one of most widely validated tests of assaying sedative and anxiolytic substances such as benzodiazepines. The test drug induced anxiolytic effect beginning at lower doses employed. An increase of most important variables of EPM test was found as follow; the percentage of time of mice spend on the open arms as well as the percentage of entries in the dark arms. The anxiolytic effect is also evidenced through light and dark test. As with the EPM test, this model is useful for modeling of anxiety. The low dose dependent effect could be attributed to biological variability, as well as chemical complexity of the test drug. Various model of anti anxiolytic testing are (23&24)

1. Elevated plus -maze model (EPM).
2. Forced swimming test (FST).
3. Light –dark test (LDT)
4. Open –field test (OFT)

1. Elevated Plus –Maze (EPM)

This has widely validated to measure anxiety to rodents. This apparatus is made of Plexiglas and consisted to two open arms (30cm x 5cm) with 30cm walls. The arms extended from the central platform (5cm x5cm). This wall is elevated 38.5cm from the room floor. The each animal is placed at the center of maze, facing one of the enclosed arms. Number of entries and time spend in enclosed and open is recorded for 5 min test. . Entry an arm is defined as animal placing all four paws onto the arm. All tests are tapped by using a video camera. After each test, the maze is carefully cleaned up with wet tissue paper (10% ethanol solution).

2. Forcing Swimming Test (FST)

The FST is most widely used pharmacological in vivo model assessing anti depressant activity. The development of immobility when mice are placed in inescapable cylinder fill with water reflects cessation of persistent escape directed behaviour (23). The apparatus consist of clear Plexiglas cylinder (20cm height x 12 cm diameter) filled to a15cm depth within the water (25ċ) in the pre-test session, every animal is placed individually into cylinder for 15 min. 24h prior to the 5min swimming test the test drug and distilled water are administered three time, immediately after 15 min pre-test,18 and 1h prior to the swimming test . During the test session a trained observer registered the immobility time, considered to be when the mouse made no further attempts to escape, apart from the movements necessary to keep its head above the water. It is suggested that the immobility reflected a state of lowered mood in which the animals had given up hope of finding an exit and had resigned themselves to the experiments situation.

3. Light Dark Test (LDT)

The apparatus consists of a Plexiglas box with two compartments (20 x 20 cm) one of which is illuminated with a white light while the other remained dark. Each animal is placed at the centered of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space is recorded for 5 min

4. Open Field Test (OFT)

Open field test area is made of acrylic transparent walls and black floor (30cmx 30cmx15cm) divided into nine square of equal area. The open field is used to evaluate the exploratory activity

of animal (25). The observed parameters are the number of square crossed (with the four paws) and number of rearing.

Antidepressant activity

Antidepressant activity was indicated the mood elevating due to various mechanism of the antidepressant drugs, such as inhibition of the enzyme of monoamine oxidase, inhibition of reuptake bioamines and enhancement of the concentration of 5-HT e) ct .Later on, inhibition of reuptake of bioamines was found to be main mechanism of action to dowenregulation of β receptor (26). Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the antidepressant This behavioural effect very similar to that found by other author after treating mice with classical antidepressant drugs as IMI (27).
Various models for antidepresent activities are as follows.

(a) Despair Swim Test
(b) Learned helplessness test
(c) Muricide behaviour in rats

1. Despair Swim Test

Principle
Behavioural despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can reduce by several agents which are therapeutically effective inhuman depression (28 & 29).

Procedure
Male Sprague-Dawley rats weighing 160–180 g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon® cages with free access to food and water. Naive rats are individually forced to swim inside a vertical. Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C). Rats placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test. Floating behaviour during this 5 min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Test drugs or standard are administered one hour prior to testing. Since experiments with the standard drug (imipramine) showed that injections 1, 5 and 24 h prior the test gave the most stable results in reducing floating these times are chosen for the experiment.

2. Learned Helplessness in Rats

Principle

Animals exposed to inescapable and unavoidable electric in one situation later fail to escape shock in a different situation when escape is possible (30). This phenomenon was evaluated as a potential animal model of depression (31 & 32).On day 19th of the investigation rats are subjected to foot shock (60 scrambled shocks ,15s duration, 0.8 mA, every min) in a two compartment jumping box with the escape door to the unelectrified adjoining compartment closed .The exercise continued for one h .On day 21, 48 hour afterwards the rates are subjected to avoidance training using the same apparatus but keeping the escape route to the un-electrified chamber open. During this avoidance training, the rats are placed in the electrified chamber allowed to acclimatize for 5 min before being subjected to 30 avoidance trials with an intertribal interval of 30s. During the first 3 s of trials a buzzer stimulus is presented followed by electro shock through the grid floor for the next 3s. The avoidance response, characterized by escape to the adjoining "safe" chamber during conditioned stimulus, is noted. Failure to escape during unconditioned stimulus within 15 s is assessed as "escape failure ".

3. Muricide Behaviour in Rats

Principle

Horovitz et al. described a selective inhibition of mouse-killing behaviour in rats by antidepressants (33). The test can be used to evaluate antidepressants such as tri-cyclic and MAO inhibitors.

Procedure

Male Sprague-Dawley rats (300–350 g) are isolated for 6 weeks in individual cages. They have access to food and water ad libitum. One mouse is placed into the rat's cage. About 10 to 30% of rats kill the mouse by biting the animal through the cervical cord. Only rats consistently killing mice within 5 min after presentation are used for the test. The mice are removed 15 to 45 s after they have been killed in order to prevent the rats from eating them. Drugs are injected i.p. to the rats before the test. Mice are presented 30, 60 and 120 min after drug administration.

Anti- Convulsant Activity

Different type of epilepsies that is grandmal, petitmal or psychomotor type can be studied in laboratory animals. Various model of Ant convulsion test are (34).

1. Pentylenetetrazole (PTZ) Seizer Test
2. Electro Shock (MES) –Induced Convulsions
3. Other Test

a. Toxicity profile
b. Effect on pentobarbital –induced sleeping time
c. Motility test
d. Amphetamine toxicity test

1. Pentylenetetrazole (PTZ) Seizer Test

Principle:

This assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that most anxiolytic agents are also able to prevent or antagonize Metrazol-induced convulsions.

Procedure
Mice of either sex with a body weight between 18 and22 g are used. The test compound or the reference drug is injected sc. or i.p. or given orally to groups of 10 mice. Another group of 10 mice serves as control. Fifteen min after sc.-injection, 30 min after i.p.-injection, or 60 min after oral administration 60 mg/kg MTZ (Metrazol) are injected subcutaneously. Each animal is placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions are recorded. At least 80% of the animals in the control group have to show convulsions.

2. Electro Shock (MES) Induced Convulsions

Principle
The electroshock assay in mice is used primarily as an indication for compounds which are effective in grand mal epilepsy. Tonic hind limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics but also by other centrally active drugs (35).
Procedure
The maximum electrical shock (MES) induced convulsion in animals represented grand mal type of epilepsy. These are type of procedures use to studies convulsions and to test to anticonvulsant drugs in laboratory animals. In MES convulsions electric shock is applied through the corneal electrode, through optic stimulation cortical excitation are produced .The MES –convulsion are divided into five phase such as Tonic flexion, Tonic extensor, Clonic convulsions, stuper, recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolished the extensor phase of MES convulsions. This procedure may be used to produce convulsions both in rat and in mice.
In this method place corneal electrodes on the cornea and apply the prescribed current and different stages of conclusions are noted as described in previous paragraph. Note the time (sec) spent by the animal in each phase of the conclusions. Inject phenytoin i.p. in rats. Wait for 30 min and subject the animals to electro-convulsions as described. Note the reduction in time or abolition of tonic extensor phase of MES convulsions.

3. Other Methods
1. Effect on pentobarbital-induced sleeping time
Rats were divided into groups [n=6] the group received dose of anticonvulsants intraperitonealy, while the control group received an equal volume of vehicle. After 10 min al animal received 50mg/kg (i.p.) of pentobarbital. The time that elapsed between loss of recovery of right reflex was taken as the seeping time and was recorded both are control and pretreated animal
2. Motility test
For locomotor activity studies, mice receiving anticonvulsant drug were placed in group of five in the rectangular case of activity meter (U. Basil, Milano). Two group of five mass each where are each dose of anticonvulsant drug 127.5 and 255mg/kg and similar vehicle treated group are used as controls .activity count are recorded at 10 min intervals for period of two hr after treatment (36) results of the test substances with that of control at each time interval and expressed as activity count of the test substances from that of control.
3. Amphetamine toxicity test
Male albino mice weighing 25-30 g are divided into four groups of 10 each. The amphetamine toxicity test is carried out as described (37). Briefly, control animals received intraperitonealy injection of the vehicle (saline) while the test animals were injected with extract. Both control and experimental animals received 5 mg/kg amphetamine 30 min later and all mice are

aggregated into cubic cages with wire mesh sides. These cages are placed in noise-controlled room at 30^0C temperature for 5 hour. At the end of this period, the number of mortality was noted and recorded.

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References

1. Seth SD. Textbook of pharmacology, published by Elsevier India private limited New Delhi 2005; 2rd edition: page no. 415.
2. Ruiz MH, Beltren YG, Mora S, Gabriela D, Glauce SB. Tortoriella J, Ramirez G. Antidepressant and anxiolytic effect of hydroalcoholic extract from *Salvia elegans* Journal of Ethnopharmacology, 2006: (article in press)
3. Suba V, Murugesan, Rao R.B, Pal M, Mandal SC, Saha B. Neuropharmacological profile of *Barleria lupulina* lindle extract in animal model. Journal of Ethnopharmacology, 2002; 81; 251-255.
4. Franco CIF, Morais LCSL, Quintans LJ,Almeida RN, Antonioui AR.. CNS pharmacological effect of the hydroalcoholic extract of *Sida cordifolia* L. leaves, Journal of Ethnopharmacology, 2005; 98: 275-279.
5. Nabeshima, T. Behavioural aspects, of cholineigic transmission: role of basal forebrain cholinergic system in memory. Process in Brain Research 1993; 98: 405-411.
6. Higashida A, Ogawa N. Differences in the acquisition process and the effect of scopolarnine on radial maze performance in the strains of rats. Pharmacology Biochemistry and Behaviour 1987; 27: 483-489.
7. Sitararn N, Weingatiner H, Gillin, JC. Human serial learning, enhancement with arecholine and choline sad impairment with scopolamine. Science 1978; 201:247-276.
8. Shaji KS, Kishore NR, Lal KP, Prince M. Revealing a hidden problem. An evaluation of a community dementia case finding program from the Indian 10/66 dementia research network. International Journal of Geriatric Psychiatry 2002; 17, 222-225.
9. Kawas C, Gray S, Brookmeyer R., Fozard J, Zonderman A. Age-specific incidence rates of Alzheimer's disease: the Baltimore longitudinal study of aging. Neurology 2000; 54, 2072-2077.
10. Vas CJ, Pinto C, Panikker D, Noronha S, Deshpande N, Kulkarni L, Sachdeva S. Prevalence of dementia in an urban Man population. International Psychogeriatrics 2001; 13: 439-450.
11. Drachman and Leavitt. Human memory and the cholinergic system. Arch neural 1974; 30: 113-121.
12. Dilts SL, and Berry CA. Effect of cholinergic drugs on passive avoidance in the mouse. J Pharmacol Exper Ther 1967; 158: 279-285.
13. Glick SD, and Zimmerberg B. The amnesic effect of scopolamine .Behav.Biol 1972; 7: 245-254
14. Schindler U, Rush DK, Fielding S. Nootropic drug: animal model for study effect on conjetion. Drugs Devel Res, 1984; 4:567-576.
15. Iverson SD. Behavioural pharmacology of dementia. Naunyn schmiedeberg's. arch pharmacol1998;288:suppl 2 R 371.
16. Darks AM, Oestreicher AB, Gispen WH, Isaakson RL. Behaviour and anatomical sequences of unilateral fornix lesion and administration of nimodipine. Brain res.1991; 557: 308-312.
17. Di Ciccod D, Anatal S, Ammassan Teule M. Prenatal to expose of gamma/neutran irridation; and sensory motor alternation and peridoxical effect on learning, teratology , 1991; 43:61-70.
18. Montgomery KC. the relation between fear induced by novel stimulation and exploratory behaviour. J Comp Physiol 1958; 48: 254-260.
19. Pellow S, Chopin PH, File SE, Briley M. Validtion of open close arm entires in an elevated plus maze as a measure antiaxiety in rats. J Neurosci Meth 1981; 14: 149-169.

20. Capaldi EJ, and Capaldi ED. Aversive learning situation; apparatus and procedure; in RD Meyer (ed) methods in psychobiology 1972; 2: 59-81.
21. Reddy DS, Kulkami, SK. The nootropic and antiamnesic effects of neurosteroids on aging and dizocilpine-induced learning impairment. Prain Remarch I 799; L 215- L 229.
22. Dhingra D, Parle M, Kulkami SK. Effect of combination of insulin with dextrose, D- (-) -fructose and diet on learning and memory in mice. Indian Journal of Pharmacology 2003; 35: 151-156.
23. Sherry A. Ferguson 1 F, Javier Cisneros B, Joseph P, Hanig, Kimberly J. B. Chronic Oral Treatment with 13-cis-Retinoic Acid (Isotretinoin) or all-trans-Retinoic Acid Does Not Alter Depression-Like Behaviors in Rats. Toxicological Sciences, 2005; 87(2): 451–459.
24. ltoh J, Nabeshima T, Kameyarna T. Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology 1990; 101: 27-33.
25. Cícero Francisco B. F, Sales K F, Barbosa1 ALDR., Bezerra JNS, Manoel A N, Marta Maria de FF, and Glauce SD BV. Alterations in behavior and memory induced by the essential oil of *Zingiber officinale* Roscoe (ginger) in mice are cholinergic-dependent. Journal of Medicinal Plants Research 2008; 2(7): 163-170,
26. Jeffrey MG, Linda CK. Litwin, and Jeffrey BM. Behavioral evidence for fl-adrenoceptor subsensitivity after subacute antidepressant $\propto 2$-adrenoceptor antagonist Naunyn-Schmiedeberg's Arch Pharmacol 1985; 329: 355- 358
27. Zafir A, Ara A, and Naheed B. *In vivo* antioxidant status: A putative target of antidepressant action Neuro-Psychopharmacology and Biological Psychiatry, 2009; 13(2): 220-228.
28. Furuzan YB, Faruk E, Güner TU, Nejat G. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology , 2000; 149:41
29. Fujimori H, Cobb D. Central nervous system depressant activity of MA 1337, 3-[3,4-m-clorophenyl-I-piperazyl)propyl)-2-qlH,3H) quinozolinedione hydrochloride. The Journal of Pharmacology and Experimental Therapeutics 1965; 148: 151-157.
30. Veronika EG, and David S. Effect of unavoidable shock on no aversively motivated performance physiological and behavior.1978; 21(6):877-881
31. Martin P, soubrie P, siman P. Noradrenergic and opioid medication of tricyclic- induced reversal of escape deficits cause by in escapable shock pretreatment in rat. Psychopharmacol, 1986; 90: 90-94.
32. Sherman MD, Allers GL, Petty F, Henna FA. A neuropharmacologically relevant animal model of depression. Neuropharmacology, 1979; 134; 265-367.
33. Onodera K, Yasumi O, and Kensuke K. Characteristics of muricide induced by thiamine deficiency and its suppression by antidepressants or intraventricular serotonin physiological and behavior.1981; 27(5):847-853.
34. Vogel H.G. "Drug Discovery and Evaluation- Pharmacological Assays", Second Completely Revised, Updated, and Enlarged Edition, published by Springer 2002, 478 -495
35. Rao SK, Andrade C, Reddy K, Madappa, KN, Thyagarajan S., Chandra S. Memory protective effect of indomethacin against electroconvulsive shock-induced rats. Biological psychiatry2002; 51: 770-773.
36. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioural and physiologic state of the mouse. Psychopharmacological 1986; 13: 222-257.
37. Hannah S, Rushton R, Tinson C. Modification of the effects of an amphetamine barbiturate mixture by the past experience of rats. Nature, 1961; 2: 533-535.