International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Preclinical Evaluation of Antidepressant Activity of New Monoamine Oxidase Inhibitors in Rodents

Aditi Panditrao[1*],Priyanka Pathak[1*],Vijaya Pandit[2*], Jayashree Dawane[3*],Sharad Bhosale[4**], Mugdha Suryawanshi[3**]

[1]Resident, [2]Professor and Head, [3]Assistant Professor, [4]Associate Professor (Retd.),
[*]Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College, Pune.
[**]Dept Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune.

Corresponding Author: Aditi Panditrao

Received: 09/08/2016 *Revised: 23/08/2016* *Accepted: 29/08/2016*

ABSTRACT

Introduction: Monoamine Oxidase Inhibitors (MAO-I) are the only antidepressants that increase levels of all three neurotransmitters implicated in depression-Serotonin, Dopamine and Norepinephrine. But, they have unfavorable side effects. Attempts were made to synthesize new MAO-Is with similar activity but better tolerability- SBK series.
Materials and Methods: The study was conducted in two phases- Screening and Evaluation. In the screening phase, eight SBK Molecules (SBK1-8) were assessed for their antidepressant potential using Tail Suspension Test (TST) in mice. Four molecules out of eight-SBK4, SBK5, SBK7, SBK8- were screened and subjected to evaluation phase using various behavioral tests- Forced Swim Test (FST) in mice, Elevated Plus Maze (EPM) in rats and Unpredictable Chronic Mild Stress (UCMS) model followed by Sucrose Preference Test (SPT) in rats. Statistical analysis was done using Graph Pad Prism 5; $p<0.05$ was significant.
Results: In the FST, the test molecules had significantly increased activity when compared to Control, however, significantly less activity as compared to Moclobemide and Fluoxetine. In EPM, activity was significantly higher compared to Control and Fluoxetine, comparable to Moclobemide and significantly less than Diazepam. Following UCMS, in the SPT, the test molecules showed significant activity as compared to Control, comparable to Moclobemide and significantly less activity than Fluoxetine.
Conclusion: The test molecules- SBK4, SBK5, SBK7 and SBK8- have good antidepressant activity. The results show that there is a latency period in their therapeutic effect, as they show better activity in the chronic model than acute model of depression.

Keywords: Tail Suspension Test; Forced Swim Test; Unpredictable Chronic Mild Stress, Sucrose Preference Test; Elevated Plus Maze.

INTRODUCTION

Depression is a chronic, hereditary condition whose etiology is largely unknown. Several hypotheses have been proposed to help understand its pathophysiology, the most widely accepted one being, the Monoamine hypothesis. According to this hypothesis, depression can be ascribed to deficits in the levels of the monoamine neurotransmitters: Serotonin (5-HT), Norepinephrine (NE) and Dopamine (DA) in the brain. [1,2] Therefore, the logical line of treatment would be to increase the amounts of these neurotransmitters by either increasing their synthesis and release or decreasing their degradation or reuptake. [2]

Out of all the antidepressant treatments discovered till now, MAO-Inhibitors (MAO-Is) are the only drugs that increase the concentrations of all three monoamines by inhibiting the MAO enzyme. However, MAO-Is, especially the non-selective ones, were found to have an unfavorable adverse effect profile as well as unpleasant food and drug interactions, especially the Tyramine reaction and thus fell into disrepute. [2,3] The selective and reversible inhibitor of MAO-A (RIMA), Moclobemide, was discovered to overcome many of the side effects its non selective predecessors suffered from. [2-4]

Extensive studies have shown that Moclobemide is comparable in efficacy to Selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs) and Tricyclic Antidepressants (TCAs). Its side effect profile is better than that of TCAs; disturbances of sleep or sexual function are negligible; there was no increase in body weight throughout a large long-term trial with Moclobemide. [2-4] Furthermore, Moclobemide lacks significant negative effects on psychomotor performance and cognition, unlike SSRIs. [3-5] However, there were a few cases of incidences of tyramine reaction even with Moclobemide. [2,4]

So, efforts were made to synthesize new monoamine oxidase inhibitors *in silico*, having activity similar to Moclobemide but with better side effect profile. Thus, the SBK series of sixteen test molecules (SBK1-16) were developed, out of which SBK1-8 were taken up for preclinical evaluation. [6]

The aim of the current study was to screen SBK series (SBK1-8) for antidepressant activity and further evaluate their activity in comparison to standard drugs like Moclobemide, Fluoxetine and Diazepam in animal models of depression.

MATERIALS AND METHODS

Animals

The animal experiments done in the present study were conducted in the Central Animal House, Bharati Vidyapeeth Deemed University Medical College, Pune. This study was conducted after getting approval from the Institutional Animal Ethics Committee (IAEC Approval no. BVDUMC/ 1540/2013-14) and all procedures were performed in compliance with CPCSEA guidelines. 86 adult albino mice weighing 18-25 g and 96 adult Wistar rats weighing 200-250 g at the start of tests were used in these studies. Housing was done in standard cages (6 animals per cage for mice, 3 animals per cage for rats) with food and water *ad libitum* while maintaining a 12-hr light-dark cycle. Animal coding was done according to standard protocol and animals were randomly allocated to different experimental groups. Tests were performed between 09:00 a.m. - 04:00 p.m. to minimize the confounding effects of circadian rhythms.

Drugs used for experiments

The test drugs, SBK series, were provided by the Department of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Paud Road, Erandwane, Pune-411038. As per instructions, the drugs were stored in air-tight tinted glass containers away from sunlight and freshly prepared. As the test drugs were insoluble in water, 1% Carboxy Methyl Cellulose was used as a suspending agent who was obtained from a local supplier. Moclobemide (50 mg/kg oral), was obtained from Intas Pharmaceuticals, Ahmedabad, with a help of a local supplier. Fluoxetine-10 mg/kg oral- (Cap. Fludac-Cadila Pharmaceuticals) and Diazepam-10 mg/kg oral- (Tab. Calmpose-Ranbaxy Laboratories Limited) were obtained from the hospital pharmacy. Sucrose (in the form of powdered sugar) was obtained from a local store.

PROCEDURES

Study design

The procedure was carried out in two phases- screening and evaluation phase.

i) Screening phase using Tail Suspension Test (TST)

60 adult albino mice were randomly allocated into 10 groups of 6 animals each - Group 1: Control (No treatment), Group 2: Moclobemide, Group 3: SBK 1, Group 4: SBK 2, Group 5: SBK 3, Group 6: SBK 4, Group 7: SBK 5, Group 8: SBK 6, Group 9: SBK 7, Group 10: SBK 8.

For this test, the test molecules were administered orally in the dose 30 mg/kg. Drugs were administered to the respective groups 1 hr before experiment. Mice were suspended from a rod on the edge of the table, 58 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail.

Recordings were taken as baseline (just before the administration of the drugs), and 1, 2, 3 and 4 hours after administration of the drugs. The period of immobility (in seconds) was recorded over a period of 5 minutes. Animals were considered immobile when they hung passively and completely motionless. [7]

Out of the 8 test molecules, four molecules-SBK4, SBK5, SBK7 and SBK8 were seen to have better antidepressant activity as compared to the control and other molecules. These molecules were taken up for further evaluation using various behavioral tests.

ii) Evaluation phase-

In this phase, 3 tests were used-

1. Forced Swim Test
2. Elevated Plus Maze
3. Unpredictable Chronic Mild Stress followed by Sucrose Preference test

Forced Swim Test (FST)

For this test, the test molecules were administered orally in two doses-30 mg/kg (SBKX) and 60 mg/kg (SBKX+). 66 adult albino mice were randomly allocated into 11 groups of 6 animals each: Group 1: Control (No treatment), Group 2: Moclobemide, Group 3: Fluoxetine, Group 4: SBK4, Group 5: SBK4+, Group 6: SBK5, Group 7: SBK5+, Group 8: SBK7, Group 9: SBK7+, Group 10: SBK8, Group 11: SBK8+.

One day prior to the experiment, naive mice were individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25°C) for 15 minutes. The animals were then removed from water, dried, and returned to their home cages. On the day of the experiment, the animals were individually forced to swim in the cylinder for a period of 5 minutes each time and the period of immobility was recorded. [7]

Recordings were taken at baseline (just before the administration of the drugs), and 1, 2, 3 and 4 hours after administration of the drugs. An animal was considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose just above the surface. [7]

Elevated Plus Maze (EPM)

For this test, the test molecules were administered orally in the dose 30 mg/kg. 48 adult Wistar Rats were randomly allocated into 8 groups of 6 animals each: Group 1: Control (No treatment), Group 2: Diazepam, Group 3: Moclobemide, Group 4: Fluoxetine, Group 5: SBK4, Group 6: SBK5, Group 7: SBK7, Group 8: SBK8.

The drugs were administered twice, 24 hrs and 1 hr before the procedure to the respective groups. The plus-maze consists of four arms with 50×10×40 cm dimensions out of which two arms are open and two are closed. Both open arms face each other and are perpendicular to the closed arms, which also face each other. The closed arms have all walls with an open roof, whereas open arms have only the base without any walls. The maze is elevated to a height of 50 cm. The rat was placed in the centre of the maze, facing the open arm opposite to the experimenter. The procedure was conducted in a dark room with a 15W bulb over the central area as the source of illumination. The observations were made from an adjacent room. An entry was recorded when all four limbs of the animal entered the arm. The apparatus was wiped with a cloth and then cleaned with ethanol soaked cotton after each animal. [7]

During the 5 min test period, the time spent in the open and closed arms were

recorded. Percentage time spent in open arm was calculated using the formula: [6]

$$\% \text{ time spent } = \frac{\text{Time spent in open arm}}{\text{Total time spent}} \times 100$$

Unpredictable Chronic Mild Stress (UCMS) followed by Sucrose Preference Test (SPT): [8,9]

For this test, the test molecules were administered orally in the dose 30 mg/kg. 48 adult Wistar Rats were randomly allocated into 8 groups of 6 animals each: Group 1: Control (Non-Stressed), Group 2: Control (Stressed), Group 3: Moclobemide, Group 4: Fluoxetine, Group 5: SBK4, Group 6: SBK5, Group 7: SBK7, Group 8: SBK8.

Animals were allowed to habituate to the sucrose solution before the UCMS protocol to establish baseline sucrose preference levels.

A slightly modified version of UCMS protocol was used for inducing anhedonia. It consisted of chronic exposure to unpredictable mild stressors over a period of 3 weeks. The stressors were: 1 hr empty bottle, 12 hr overnight illumination, 24 hr separation, 24 hr high density housing (6 rats per cage), 10 min inescapable shock (1.5mA, 15 s on, 150 s off), 10 min cold swim at 4°C, 10 min tail pinch, 24 hr cage tilt at 45°, 12 hr soiled cage. Immediately after each stress session, the rats were returned to the cage and maintained in standard conditions until the next stressor was given. All groups, except for Group 1, were subjected to UCMS. During the last 1 week of UCMS, animals were given daily treatments as per the groups. [8,9]

To test sucrose preference, animals that were food and water-deprived for 18h, were presented with two pre-measured bottles one with 1% sucrose solution and other with water for a period of 24 hrs. Sucrose preference was calculated according to the formula: [8,9]

$$\text{Sucrose Preference} = \frac{\text{Sucrose intake}}{\text{Total Fluid intake}} \times 100$$
$$(\text{Sucrose intake} + \text{Water intake})$$

The test was carried out three times - before beginning the UCMS protocol, at the end of the second week and at the end of the stress protocol (i.e. the third week). After recording the amount of sucrose solution and water consumed, sucrose preference for all the groups was calculated.

Statistical analysis

Results were expressed as Mean ± Standard Deviation (SD). For Tail Suspension Test, Forced swim test and Sucrose Preference Test, repeated measures ANOVA followed by post hoc Tukey's test was done. For Elevated Plus Maze, one way ANOVA followed by post hoc Tukey's test was done. Significance was accepted for $P < 0.05$. All statistical analyses were carried out using Graph Pad Prism version 5.03 (San Diego, CA).

RESULTS

Screening Using Tail Suspension Test (TST)

Immobility was recorded in seconds. All results were compared to Control and Moclobemide. All molecules, except SBK3 showed significant activity ($p<0.05$) in comparison to Control. As is seen in Fig. 1, all animals showed similar baseline readings. In comparison to Moclobemide, however, the activity was significantly less ($p<0.05$). Significant reduction in immobility was seen at the end of 2 hrs- Table I. Out of the eight molecules, most significant activity was seen with SBK4, SBK5, SBK7 and SBK8. Fig. 2 shows the results of comparison of antidepressant activity of SBK4 with control and other test molecules. SBK4 was the first test molecule in the SBK series to show good antidepressant activity as compared to the control. Hence, all other molecules were compared to it for evaluation of antidepressant activity. It is evident in Fig 2 that SBK1, SBK2, SBK3 and SBK6 had significantly less antidepressant activity ($p<0.001$) as compared to SBK4. The activity of SBK5 was also significantly less, but significance was at $p<0.01$. SBK7 and SBK8 showed no statistical significance and were comparable in efficacy to SBK4 ($p>0.05$). So, SBK4, SBK5, SBK7 and

SBK8 were considered for further evaluation.

Table I: Period of immobility (in secs.) using TST

Group	Baseline	After 1 hr	After 2 hrs	After 3 hrs	After 4 hrs
C	136.3±5.4	148.8 ± 2.6§	169.3 ± 9.6§	174.5±5.3§	181.3 ± 3.0§
M	137.2±7.9	94.3 ± 2.7*	56.0 ± 5.3*	59.8 ± 1.9*	73.0 ± 4.4*
S1	136.7±7.8	132.0±2.0*§	129.0±4.4*§	130.8±1.8*§	127.5±3.2*§
S2	137.8±8.9	133.5±2.5*§	128.3±3.3*§	133.7±2.0*§	124.0±3.5*§
S3	134.3±9.7	150.8 ± 2.4§	171.0 ± 3.8§	163.8±2.4*§	152.7±3.3*§
S4	136.8±4.7	115.7±4.0*§	72.0 ± 4.6*§	80.6 ± 5.0*§	85.33±2.5*§
S5	139.2±7.3	115.5±3.2*§	83.3 ± 3.5*§	85.0 ± 2.8*§	86.8 ± 3.4*§
S6	140.7±6.6	133.2±3.4*§	111.8±6.5*§	122.5±3.6*§	134.7±4.2*§
S7	141.7±7.1	103.0±3.8*§	66.8 ± 2.4*§	73.3 ± 3.3*§	79.5 ± 1.8*§
S8	138.0±6.8	108.0±3.6*§	78.8 ± 2.3*§	82.3 ± 2.7*§	94.5 ± 2.4*§

Data given are Mean ± Standard Deviation (SD)

Repeated measures ANOVA with post hoc Tukey's test were used to compare mean differences between control, standards and test groups. p value <0.05 considered statistically significant. *: comparison with control was significant (p<0.05), §: comparison with Moclobemide was significant (p<0.05), C: Control; M: Moclobemide; S1: SBK1; S2: SBK2; S3: SBK3; S4: SBK4; S5: SBK5; S6: SBK6; S7: SBK7; S8: SBK8

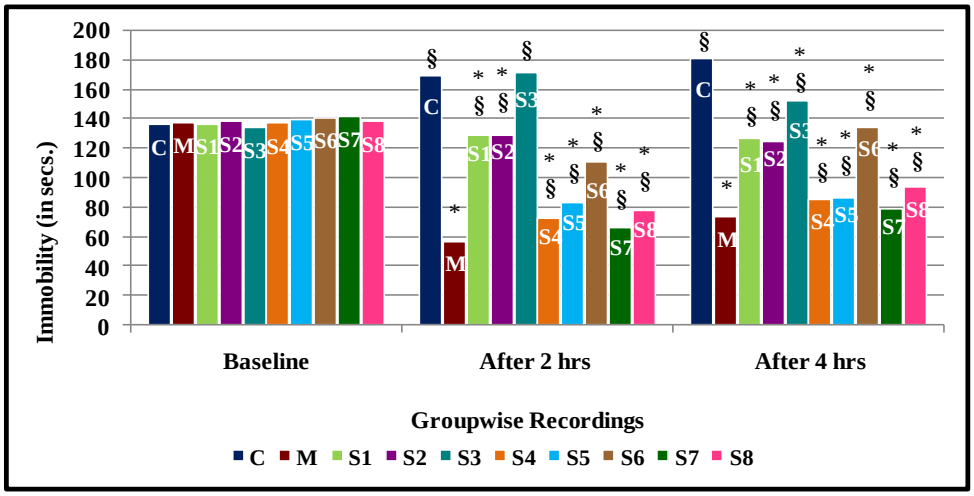

Fig. 1: Period of immobility (in secs.) using TST

Data given are Mean ± Standard Deviation (SD). Repeated measures ANOVA with post hoc Tukey's test was used to compare mean differences between control, standard and test groups. *: comparison with control was significant (p<0.05), §: comparison with Moclobemide was significant (p<0.05). C: Control; M: Moclobemide; S1: SBK1; S2: SBK2; S3: SBK3; S4: SBK4; S5: SBK5; S6: SBK6; S7: SBK7; S8: SBK8

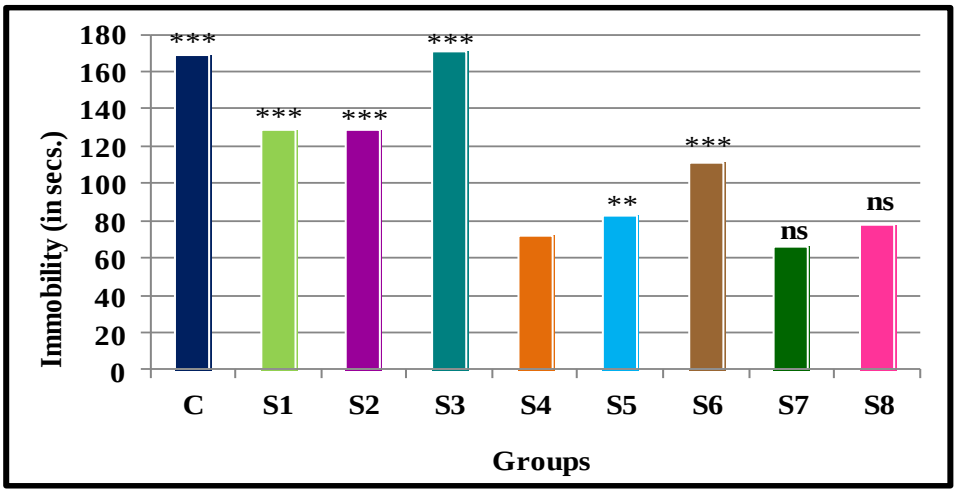

Figure 2: Period of immobility (in secs.) in TST – After 2 hrs

Data given are Mean ± Standard Deviation (SD). Data from Repeated measures ANOVA with post hoc Tukey's test at 2 hrs was used to compare results of SBK4 with Control and other test molecule groups. ***: comparison with SBK4 was significant (p<0.001),**: comparison with SBK4 was significant (p<0.01),*: comparison with SBK4 was significant (p<0.05),ns: comparison with SBK4 was not significant (p>0.05),C: Control; S1: SBK1; S2: SBK2; S3: SBK3; S4: SBK4; S5: SBK5; S6: SBK6; S7: SBK7; S8: SBK8

Forced Swim Test: Immobility was recorded in seconds. As is seen in Fig. 3, all animals showed similar baseline readings. All results were compared to Control, Moclobemide and Fluoxetine. The test molecules showed significant antidepressant activity ($p<0.05$) as compared to Control. In comparison to Fluoxetine and Moclobemide, they showed significantly less activity ($p<0.05$). The test molecules SBK4, SBK5, SBK7 and SBK8 were tested at 2 doses-30mg/kg and 60 mg/kg. Both doses were equi-effective ($p>0.05$) - Fig 4.

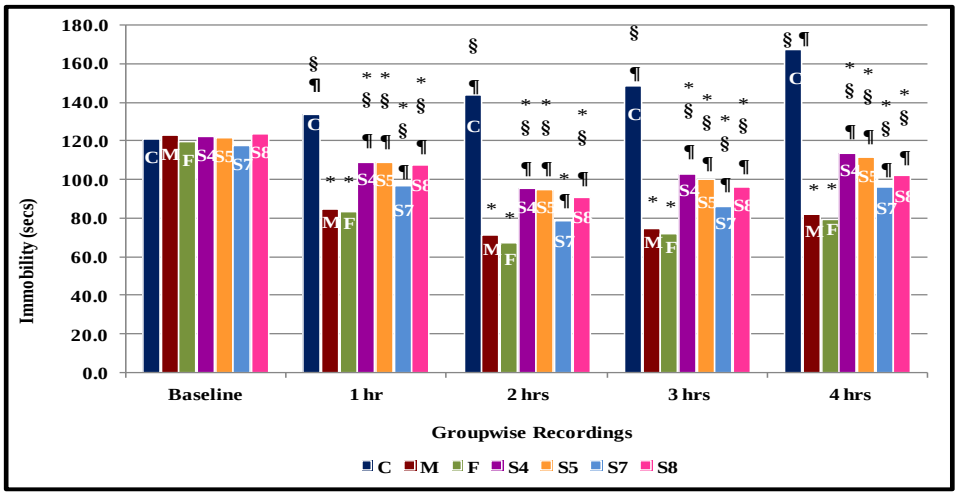

Fig. 3: Period of Immobility (in secs.) using Forced Swim Test

Data given are Mean ± Standard Deviation (SD). Repeated measures ANOVA with post hoc Tukey's test were used to compare mean differences between control, standards and test groups. *: comparison with control was significant ($p<0.05$), §: comparison with Moclobemide was significant ($p<0.05$), ¶: comparison with Fluoxetine was significant ($p<0.05$). C: Control; M: Moclobemide; F: Fluoxetine; S4: SBK4; S5: SBK5; S7: SBK7; S8: SBK8

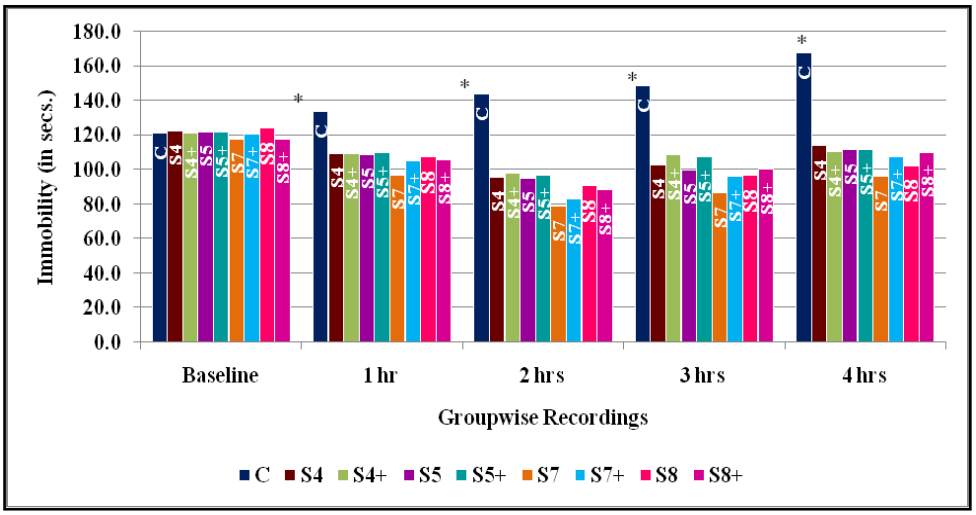

Fig. 4: Period of Immobility (in secs.) between two dose groups

Data given are Mean ± Standard Deviation (SD). Repeated measures ANOVA with post hoc Tukey's test were used to compare mean differences between control and test groups.

*: comparison with control was significant ($p<0.05$), C: Control; S4: SBK4 (30 mg/kg); S4+: SBK4 (60 mg/kg); S5: SBK5 (30 mg/kg); S5+: SBK5 (60 mg/kg); S7: SBK7 (30 mg/kg); S7+: SBK7 (60 mg/kg); S8: SBK8 (30 mg/kg); S8+: SBK8 (60 mg/kg)

Elevated Plus Maze: The anti-anxiety activity was compared using percentage time spent in the open arms. All results were compared to Control, Diazepam, Moclobemide and Fluoxetine. As is seen in Fig.5, all test molecules showed significant activity ($p<0.05$) as compared to the Control group. However, they were significantly less effective ($p<0.05$) than Diazepam. Compared to Moclobemide, SBK4, SBK5 and SBK7 showed comparable activity ($p>0.05$), whereas SBK8 showed

significantly less activity ($p<0.05$). Fluoxetine did not show significant activity ($p>0.05$) as compared to Control. The test molecules showed significantly more anti-anxiety compared to Fluoxetine ($p<0.05$). Out of all the test molecules, the best anti-anxiety activity was seen with SBK7.

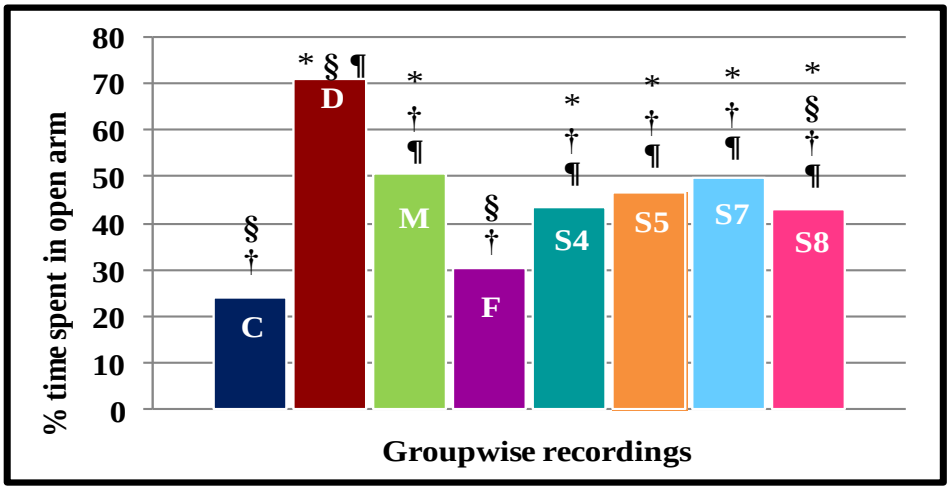

Fig. 5: Percentage Time Spent in Open Arm

Data represented as Mean ± Standard Deviation (SD). One way ANOVA followed by post hoc Tukey's test was done to compare differences between control, standards and test groups. p value <0.05 considered statistically significant

*: comparison with Control was significant ($p<0.05$), †: comparison with Diazepam was significant ($p<0.05$), §: comparison with Moclobemide was significant ($p<0.05$), ¶: comparison with Fluoxetine was significant ($p<0.05$)

C: Control; D: Diazepam; M: Moclobemide; F: Fluoxetine; S4: SBK4; S5: SBK5; S7: SBK7; S8: SBK8

Preference for Sucrose Solution following Unpredictable Chronic Mild Stress

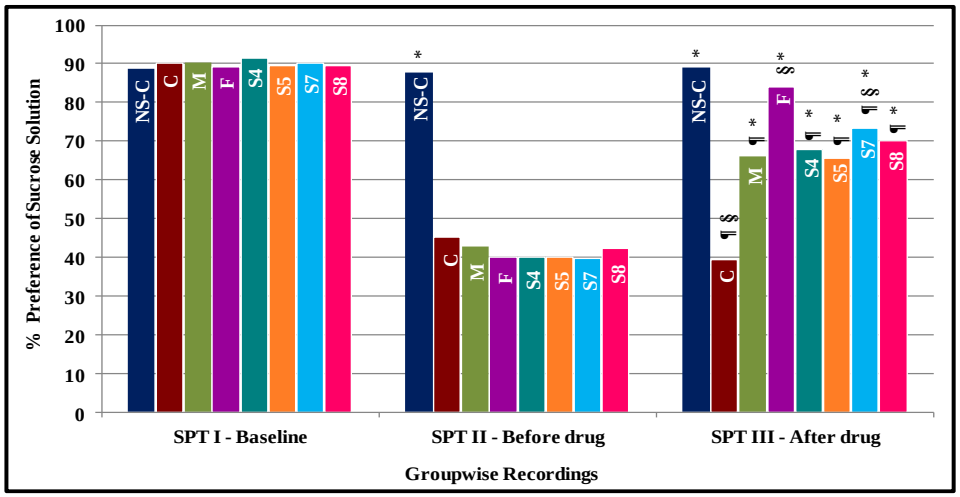

Fig 6: Preference for Sucrose Solution following Unpredictable Chronic Mild Stress (UCMS) –

Data represented as Mean ± Standard Deviation (SD). Repeated measures ANOVA with post hoc Tukey's test were used to compare mean differences between NS-Control, S-Control, standards and test groups. *: comparison with Stressed Control (C) was significant ($p<0.05$), §: comparison with Moclobemide was significant ($p<0.05$), ¶: comparison with Fluoxetine was significant ($p<0.05$)All groups except NS-C were subjected to stress., NS-C- Non-stressed Control; C- Stressed Control; M -Moclobemide; F-Fluoxetine; S4-SBK4; S5- SBK5; S7 - SBK7; S8- SBK8; SPT- Sucrose Preference Test.

Baseline levels were recorded before starting the UCMS (SPT I - Baseline). These were found to be comparable ($p>0.05$) between all the groups. At the end of the second week (SPT II), the preference for sucrose had significantly decreased ($p<0.05$) in all the Stressed animals as compared to the Non-Stressed Control animals. By the end of third week (SPT III), all treated groups showed increased sucrose preference as compared to Stressed Control group (C), though it was still significantly less ($p<0.05$) than that of Non-stressed Control group (NS-C). As observed in Fig.

6, all the test molecules showed significant activity ($p<0.05$) as compared to the Stressed Control (C) group. In comparison to Fluoxetine, the test molecules showed significantly less activity ($p<0.05$). In comparison to Moclobemide, SBK7 showed significantly higher ($p<0.05$) activity, whereas SBK4, SBK5 and SBK8 showed comparable ($p>0.05$) activity. Out of all the test molecules, maximum antidepressant activity was shown by SBK7.

DISCUSSION

Depression is a disease known since time immemorial but whose etiology is still unknown For a long time, the standard therapy included use of drugs like TCAs and MAO-Is, but these fell into disrepute because of their side effects. [2,4,10] The current antidepressant therapy includes use of SSRIs and SNRIs. However, these agents have their adverse effects too. [2,5,10,11] Also, in cases where these first line agents fail, like refractory depression, MAO-Inhibitors are usually used. [2-4] Being equally effective to other antidepressants like SSRIs and with favourable adverse effect profile, Reversible Inhibitor of MAO-A, Moclobemide is clinically preferred to the non-selective MAOIs. [2,4]

The test molecules (SBK series) evaluated in the present study were synthesized with the aim to develop agents interacting with MAO-A enzyme which would have efficacy similar to Moclobemide, but would be devoid of its adverse effect profile, especially the tyramine reaction. [6]

Using computer software PHASE, functional groups necessary in a compound for interaction with the MAO-A enzyme were identified. Protein Database (PDB-2Z5X) was searched for and eleven compounds having these functional groups were found. With the help of computer software Glide, docking studies were done to see the interaction of the compounds with the enzyme. Out of the eleven compounds, one compound which showed maximum active interaction with the enzyme and which could be easily processed and structurally modified in the laboratory was then taken up for further development. This was a Benzimidazole derivative. A series of sixteen molecules were then developed from this compound and named the SBK series. These molecules were synthesized by performing many chemical reactions on the parent molecule. [6]

In the current study, the eight molecules, SBK1-8 were screened for antidepressant activity using Tail Suspension Test for detection of antidepressant potential. Out of eight, four molecules showed promising results-SBK4, SBK5, SBK7, and SBK8. These molecules were then taken up for evaluation using various models of depression.

Mimicking any behavioural trait in animals is extremely difficult because they lack self-consciousness, self-reflection and aspects of the disorder such as depressed mood, low self-esteem or suicidality. [12,13] So, in depression as well as other mood disorders, certain endophenotypes are considered which can independently be evaluated in animals. An endophenotype is an internal phenotype which is easily measurable and lies between the genes and the disease process. [14] Endophenotypes used for evaluating depression are various behavioural changes produced in animals, mimicking clinical manifestations of the disease. These include anxiety, reward, social and despair-based behaviour among others. [13-15]

Evidence has shown that stress (acute and chronic) is one of the main risk factors in the etiopathogenesis of depression. Exposure to stress or to traumatic life experiences has a strong impact on the manifestation of depression, suggesting an impairment of proper stress-coping mechanisms in depressed patients. Therefore, depression is also regarded as a stress-related disorder and accordingly, many of the animal models of depression are based on the exposure to various types of acute or chronic stressors. [12,13,16] Therefore, the present study was conducted

using behavioral tests like Tail Suspension Test and Forced Swim Test that use acute stress to test antidepressant activity, and UCMS that induces depression by chronic stress.

The Tail Suspension Test (TST), (Steru et al. 1985), is a commonly used test to screen potential antidepressants. TST is based on the observation that mice, after initial escape-oriented movements, develop immobility when placed in an inescapable stressful situation- the hemodynamic stress of being hung in an uncontrollable fashion by their tail. Acute antidepressant treatments decrease the immobility scores. [7,17] TST is performed on mice and not rats, as rats are heavier and being hung by the tail could lead to the stripping or fracture of the tail. [18]

Tian et al. (2014) assessed the antidepressant activity of novel antidepressant Adhyperforin using the TST. [19] Mishra et al (2013) assessed the antidepressant activity of Eclipta alba using tail suspension test. [20] In 2009, Ismail et al used TST to compare activities of Moclobemide and Fluoxetine and found them to be equi-effective. [21]

In the current study, the test molecules were screened for their antidepressant potential in comparison to a Control group as well as Moclobemide. All the molecules, except SBK3, showed significant activity ($p<0.05$) when compared with Control, but significantly less as compared to Moclobemide. Highest activity was seen at 2 hours and remained mostly stationary or slightly decreased at the end of 4 hours- Table I. SBK4, SBK5, SBK7 and SBK8 show considerable antidepressant activity in comparison to other SBK molecules. This effect is better appreciated in Fig. 1 and 2. SBK4 was the first test molecule in the SBK series to show good antidepressant activity as compared to the control. Hence, all other molecules were compared to it for evaluation of antidepressant activity.

Thus, SBK4, SBK5, SBK7 and SBK8, which showed most significant activity out of all the test molecules, were taken up for further evaluation.

The evaluation of the antidepressant activity of the 4 SBK molecules was done using three tests -

1. Forced Swim Test (FST)- to assess the despair based or stress coping behavior
2. Elevated plus maze (EPM)-to assess the anti-anxiety activity
3. Unpredictable Chronic Mild Stress (uCMS) followed by Sucrose Preference test (SPT)- to assess Reward related and anhedonic behavior

The Forced Swim Test (FST), (Porsolt et al. 1977), is the most widely used laboratory test for assessing the potential antidepressant activity of drugs. It has the ability to detect a broad spectrum of clinically effective antidepressants and it is a fast method which can meet the high-throughput demands of the pharmaceutical industry. It was originally developed to screen monoamine-based antidepressants. [7,14 ,22]

FST assesses the response to an acute inescapable stressor, provoking despair-based behavior or a stress coping behavior in the form of immobility. The FST makes use of the fact that rodents will eventually develop immobility when being placed in a cylinder of water after they have stopped active escape behaviors, such as climbing or swimming. If they are replaced in the testing apparatus repeatedly, they resume this posture quickly, thus demonstrating helplessness. This helplessness, in the form of immobility is a failure of the willingness to continue the escape behavior-Behavioral Despair. Antidepressants reduce the tendency of development of such despair. Animals given acute doses of antidepressants show escape-oriented behaviors than their no-treatment counterparts. *This test, effectively, personifies the feeling of despair or helplessness experienced by depressed individuals.* [17,14,22]

FST has strong predictive validity, good reliability, some face validity and poor construct validity. [22] The antidepressant

activity of the new SNRI Vortioxetine was assessed by the authors Mork et al. in 2012, Li et al. in 2013 and Jensen et al. in 2014 using the Forced Swim test. [23] In another study, the antidepressant potential of novel antidepressant Adhyperforin was assessed using the FST by Tian et al. in 2014. [19] Shin et al in 2014 used FST in their study to assess antidepressant activity of another novel polyherbal formulation Radix Polygalae. [24]

Cryan et al (2005) compared the antidepressant activities of Moclobemide, Reboxetine and Fluoxetine using a modified FST and found these drugs to be equi-effective with respect to each other. [25] In 2009, Ismail et al. used FST to compare the activities of Moclobemide, Fluoxetine and Phenelzine and found all three drugs comparable to each other. [21]

In the current study, the antidepressant activity of the test molecules was evaluated in two doses 30 mg/kg and 60 mg/kg using the FST. The selection of doses of the test molecules SBK (30 mg/kg) was based on the equivalent dose of Moclobemide 50 mg/kg found by 3D-QSAR, and its double dose (60 mg/kg). All the test molecules were equally effective at both the doses (Fig. 4). Based on this result, the decision was made to use only the dose of 30 mg/kg for the other tests. It was already stated in the protocol of the study at the time of ethical approval, that the two doses would be compared and if no difference in activity is found, or if there is mortality due to double dose (60 mg/kg), the further tests would be done using only dose of 30 mg/kg. Comparison of antidepressant activity was done with the Control group, Moclobemide and Fluoxetine. Fluoxetine was added because it is the first line of treatment of Major Depressive Disorder in current clinical practice. It was seen that all four test molecules had significant antidepressant activity ($p<0.05$) as compared to the Control group, but were significantly less effective ($p<0.05$) than Moclobemide and Fluoxetine (Fig. 3). The immobility seen in the Control animals progressively increased with time- (Fig. 3 and 4). This is because of the helplessness as a result of the Behavioral Despair, which is the basis of this test. Moclobemide and Fluoxetine had comparable activity ($p>0.05$). This result is similar to results of Cryan et al (2005) [25] and Ismail et al (2009). [21] Maximum activity was seen at 2 and 3 hours after the drug administration (Fig. 3). Out of the four molecules tested, SBK7 showed maximum antidepressant activity.

Anxiety is a symptom commonly present in depressed individuals. Many animal studies have shown that antidepressants also show anti-anxiety activity. Antidepressants may not be effective in typical anxiety, but for anxiety manifested in depression, antidepressants may be useful. [13,26,27] The elevated plus maze (EPM) is the most commonly employed animal model of anxiety in current practice. The apparatus is raised above floor level and is composed of two closed arms perpendicular to two open arms. The test exploits the natural tendency of rodents to explore novel environments and their innate avoidance of unprotected, bright and elevated places (represented by the open arms). [7,28] Routinely, when an animal is placed on the maze, initially it explores the open arm, but eventually goes to the closed arm. Administration of classical anti-anxiety drugs, such as Diazepam, increases exploration of the open arms and thereby the time spent in the open arms. [26-28]

Parise et al. (2013) and Autry et al. (2011) used the Elevated plus maze for assessment of anti-anxiety activity of Ketamine. [29] Mak et al (2011) used the EPM to test anti-anxiety activities of novel peptidic oxytocin and vasopressin receptor ligands. [30] Mansouri et al (2013) used this model to evaluate anti-anxiety activity of Gallic Acid. [31]

The anti-anxiety activity of Moclobemide was evaluated by Nowakowska et al (1998) using the EPM and showed that Moclobemide has

significant anti-anxiety activity as compared to control. [32] In another study, Eroglu et al (1998) showed that Moclobemide in both acute and subchorionic doses was able to prevent yohimbine-induced anxiety in rats. [33]

Silva et al (1999) studied the effects of fluoxetine on anxiety using EPM and found that both acute and chronic fluoxetine had anxiogenic activity. [34] Kurt et al (2000) evaluated the antianxiety potential of Sertraline and fluoxetine in comparison to Diazepam and found that both the SSRIs had anxiogenic activities. [35]

In the current study, the test molecules were compared to Control group, Diazepam, Moclobemide and Fluoxetine. Diazepam is a standard anti-anxiety drug used in clinical practice and was a reference standard for this model. As illustrated in Fig. 5, all four test molecules significantly increased ($p<0.05$) the time spent in the open arms as compared to the control group. But this increase was not as significant as Diazepam ($p<0.05$). However, the anti-anxiety activities of SBK4, SBK5 and SBK7 were comparable to that of Moclobemide ($p>0.05$). (Fig. 5) Fluoxetine did not show anti-anxiety activity ($p>0.05$). This result was similar to the results seen in studies done by Silva et al (1999) and Kurt et al (2000). However, in the current study, while Fluoxetine did not produce anti-anxiety effects, it was not anxiogenic. The results seen with Moclobemide in this study are similar to the results seen in studies done by Nowakowska et al (1998) [32] and Eroglu et al (1998). [33] Of all the test drugs, SBK7 showed maximum anti-anxiety activity.

The Forced Swim Test and Elevated Plus Maze employ acute stress and an acute dose of the drug to evaluate the antidepressant activity. However, clinical depression is a chronic condition, which requires long term administration of the antidepressant drug (more than 3 weeks). Also, chronic stress is considered to be an important factor in the development of depression. [13,36] Therefore, it is important to employ a test which would induce and measure the chronic nature of depression and the effect of repeated use of the drugs. Battery of tests for antidepressant assessment cannot be complete without the inclusion of Unpredictable Chronic Mild Stress (UCMS) in their protocol. [13]

The Unpredictable chronic mild stress (UCMS) model, originally developed by Katz et al. and later perfected by Willner et al. (1987), was aimed to simulate stressful situations to induce behavioral changes in rodents that closely resemble anhedonic symptoms seen in human depression. [8] Anhedonia is one of the core symptoms of Major Depression. [37]

UCMS model involves exposing rodents to a series of repeated unpredictable physical stressors, including foot shock, low temperatures, overcrowding, and water and food deprivation etc, over a period of few weeks. These stressors should be unpredictable, uncontrollable and challenge the natural defense mechanisms of the animal. Repetition of a certain stressor may make the model predictable and lead to adaptation to the stressor instead of inducing stress-coping behaviors or helplessness. By the second week, animals develop a decrease in reward sensitivity or anhedonia. This anhedonia can be reverted by chronic, but not acute, administration of antidepressants. [8,9,13] The measurement of anhedonia can be done by tests like the Sucrose Preference Test (SPT), Sucrose Consumption Test, Intracranial Self Stimulation, Conditioned Place-Preference test, Female Urine Sniffing Test etc. [13] The model has good predictive, face and construct validity. [8,13]

In 1993, Moreau et al. evaluated the anti-anhedonic activity of Moclobemide using the UCMS model followed by Intracranial Self Stimulation and found that Moclobemide significantly reversed anhedonia after 2 weeks of administration. [38] In 2004, Ossowska et al. conducted a study using the UCMS model followed by assessment of fighting behavior of rats with Imipramine, Mianserin, Fluoxetine, Moclobemide, Tianeptine and found that all

these drugs significantly reversed the anhedonia. [39] In 2003, Ducottet et al. used this model to study a new drug Antalarmin vs. Fluoxetine, where it was found that Fluoxetine had significantly more anti-anhedonic activity than Antalarmin. [40]

Sucrose drinking is the most commonly utilized assay to assess the impact of UCMS, in the form of Sucrose Preference Test (usually done in rats) or Sucrose Consumption Test (mainly done in mice). UCMS-exposed animals show deficits in their motivation to consume a dilute (1-2%) solutions of sucrose measured as a preference of the sucrose solutions against water. Rodents have a natural preference for weakly sweetened solutions of sucrose. [8,13] Animals previously habituated to sucrose are typically given a choice of drinking sucrose versus water in a two-bottle test. While non-stressed animals typically show a preference for drinking weak sucrose solutions, animals exposed to UCMS tend to lose this preference. [8,9]

Li et al. (2011), Parise et al. (2013) and Ma et al. (2013) evaluated anti-anhedonic activity of Ketamine using the UCMS model followed by Sucrose Preference test. [29] Tian et al. (2014), assessed the anti-anhedonic activity of novel antidepressant Adhyperforin using the UCMS followed by SPT. [19]

In this study, UCMS protocol was implemented for 3 weeks. SPT (I) was done in the beginning to establish baselines. SPT (II) was done at the end of the second week to assess whether anhedonia was induced. Along with stressor, respective drug treatment was given daily in the last week. SPT (III) was done at the end of the third week to assess effect of drug. The test drugs were compared against a Non-Stressed Control, Stressed Control, Moclobemide and Fluoxetine groups. As is evident in Fig. 6, in the Stressed Control group, sucrose preference persistently remained significantly less ($p<0.05$) as compared to Non-Stressed Control group. All test molecules had significant activity ($p<0.05$) as compared to the Stressed Control group. The standard drug Fluoxetine almost completely reversed anhedonia and brought the sucrose preference levels equal to the non-stressed animals ($p>0.05$). All the test molecules had less activity as compared to Fluoxetine ($p<0.05$). It was seen that SBK7 had more antidepressant activity than all other test molecules and the standard drug Moclobemide ($p<0.05$). SBK8 also showed better activity than Moclobemide, but this was not statistically significant. SBK4 and SBK5 had activity comparable to Moclobemide ($p>0.05$) (Fig. 6).

No mortality of animals or changes/loss of fur was recorded over the course of the study. This study was a proof of activity study and thus, side effect profile and toxicity tests were not undertaken.

CONCLUSION

The current study was a preclinical evaluation of the antidepressant activity of new molecules, the SBK series. In the current study, all test molecules exhibited significant antidepressant activity as compared to Control. In acute stress and single dose tests SBK molecules were less effective than both Moclobemide and Fluoxetine. In chronic stress and multiple dose model, the test molecules were equi-effective (SBK4 and SBK5) or more effective (SBK7 and SBK8) than Moclobemide, though less effective than Fluoxetine. This suggests a latency period in the onset of clinical effect of the test molecules. Amongst all test molecules, SBK7 showed maximum antidepressant activity.

Further studies including acute, subchorionic and chronic toxicity studies, interactions with tyramine have been planned to establish the safety profile of the test molecules. Studies to see the biochemical, neuroendocrine and neuroanatomical effects of the test molecules need to be undertaken before they can be introduced in human trials.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of the Central Animal House, BVDUMC, Pune for their co-operation during this study. Heartfelt thanks to Dr. Mridul Panditrao and Dr. Minnu Panditrao for their intellectual support during the writing of this manuscript.

REFERENCES

1. Hindmarch I. Expanding the horizons of depression: beyond the monoamine hypothesis. Human Psychopharmacology: Clinical and Experimental. 2001;16(3):203-218
2. Goodman L, Brunton L, Chabner B, Knollmann B. Goodman & Gilman's pharmacological basis of therapeutics. New York: McGraw-Hill; 2011:397-416.
3. Bonnet U. Moclobemide: Evolution, Pharmacodynamic, and Pharmacokinetic Properties. CNS Drug Reviews. 2002;8(3): 283-308
4. Bonnet U. Moclobemide: Therapeutic Use and Clinical Studies. CNS Drug Reviews. 2003;9(1):97-140
5. Ferguson J. SSRI Antidepressant Medications. Prim Care Companion J Clin Psychiatry. 2001;03(01):22-27
6. Shelke S, Bhosale S, Dash R, Suryawanshi M, Mahadik K. Exploration of new scaffolds as potential MAO-A inhibitors using pharmacophore and 3D-QSAR based in silico screening. Bioorganic & Medicinal Chemistry Letters. 2011;21(8):2419-2424
7. Vogel H, Mass J, Gebauer A. Drug discovery and evaluation. Berlin: Springer; 2011.
8. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology. 1987; 93(3):358-364
9. Willner P. Chronic Mild Stress (CMS) Revisited: Consistency and Behavioural-Neurobiological Concordance in the Effects of CMS. Neuropsychobiology. 2005; 52 (2):90-110.
10. Schildkraut J. The Catecholamine Hypothesis of Affective Disorders: A Review of Supporting Evidence. American Journal of Psychiatry. 1965; 122(5):509-522.
11. Bunney W. Norepinephrine in Depressive Reactions. Arch Gen Psychiatry. 1965; 13 (6):483-494.
12. Nestler E, Hyman S. Animal models of neuropsychiatric disorders. Nature Neuroscience. 2010;13(10):1161-1169
13. Dedic, Nina, Sandra M. Walser, and Jan M. Deussing. Mouse models of depression. Psychiatric Disorders-Trends And Developments. 2011; 1:185-221
14. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psycho-pharmacology. 2004; 177(3):245-255.
15. Hasler G. Discovering Endophenotypes for Major Depression. Neuro psychopharmacology. 2004; 29:1765-81.
16. Abelaira H, Reus G, Quevedo J. Animal models as tools to study the pathophysiology of depression. Rev Bras Psiquiatr. 2013; 35:S112-S120.
17. Cryan, John F., Cedric Mombereau, and Annick Vassout. "The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice." Neuroscience & Biobehavioral Reviews 29.4 (2005): 571-625.
18. Thierry B, Steru L, Simon P, Porsolt R. The tail suspension test: Ethical considerations. Psychopharmacology. 1986;90(2):284-285
19. Tian J, Zhang F, Cheng J, Guo S, Liu P, Wang H. Antidepressant-like activity of adhyperforin, a novel constituent of Hypericum perforatum L. Sci Rep. 2014;4.
20. Mishra S, Jena M, Pal A. Evaluation Of Antidepressant Activity Of Eclipta Alba Using Animal Models. Asian J Pharm Clin Res. 2013; 6(3):118-120.
21. Ismail, M. Owais, and Ahsana Dar. Comparison of the Efficacy of Fluoxetine, Phenelzine and Moclobemide in Rodents Using Animal Models of Depression. Pak J of Pharmacol. 2009; 26(2):19-23.
22. Cryan J, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in Pharmacological Sciences. 2002; 23(5):238-245.
23. Sanchez C, Asin K, Artigas F. Vortioxetine, a novel antidepressant with multimodal activity: Review of preclinical and clinical data. Pharmacol & Therap. 2015; 145:43-57.

24. Shin I, Son S, Park H, Kim Y, Park S, Swanberg K et al. Preclinical Evidence of Rapid-Onset Antidepressant-Like Effect in Radix Polygalae Extract. PLoS ONE. 2014; 9(2):e88617.
25. Cryan J, Page M, Lucki I. Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. Psychopharmacology. 2005; 182(3):335-344.
26. Carobrez A, Bertoglio L. Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. Neuroscience & Biobehavioral Reviews. 2005; 29(8):1193-1205.
27. Campos A, Fogaca M, Aguiar D, Guimaraes F. Animal models of anxiety disorders and stress. Rev Bras Psiquiatr. 2013; 35:S101-S111.
28. Ruarte M, Alvarez E. Behavioral profiles displayed by rats in an elevated asymmetric plus-maze: effects of diazepam. Braz J Med Biol Res. 1999; 32(1):99-106.
29. Browne C, Lucki I. Antidepressant effects of ketamine: mechanisms underlying fast-acting novel antidepressants. Frontiers in Pharmacology. 2013; 4.
30. Mak P, Broussard C, Vacy K, Broadbear J. Modulation of anxiety behavior in the elevated plus maze using peptidic oxytocin and vasopressin receptor ligands in the rat. Journal of Psychopharmacology. 2011; 26(4):532-542.
31. Mansouri M, Soltani M, Naghizadeh B, Farbood Y, Mashak A, Sarkaki A. A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze. Pharmacology Biochemistry and Behavior. 2014; 117:40-46.
32. Nowakowska E, Choderna A, Kus K, Rybakowski J. Anxiolytic and memory improving activity of moclobemide. European Psychiatry. 1997; 12:214s-214s.
33. Eroğlu L, Güven Ö. The Effects of Moclobemide on the Yohimbine-Induced Anxiogenic Action in the Elevated Plus-Maze. Pharmacological Research. 1998; 37(2):137-143.
34. Silva M, Alves C, Santarem E. Anxiogenic-like effect of acute and chronic fluoxetine on rats tested on the elevated plus-maze. Braz J Med Biol Res. 1999; 32(3):333-339.
35. Kurt M, Arik A, Çelik S. The Effects of Sertraline and Fluoxetine on Anxiety in the Elevated Plus-Maze Test. Journal of Basic and Clinical Physiology and Pharmacology. 2000; 11(2)173-180.
36. Krishnan V, Nestler E. The molecular neurobiology of depression. Nature. 2008; 455(7215):894-902.
37. Association A. Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5®). Washington, D.C. American Psychiatric Publishing; 2013.
38. Moreau J, Jenck F, Mutel V, Martin J, Haefely W. Evidence for a role of 5-HT1C receptors in the antiserotonergic properties of some antidepressant drugs. European Journal of Pharmacology. 1993; 231(2):223-229.
39. Ossowska, Grażyna, et al. Antidepressants in chronic unpredictable mild stress (CUMS)-induced deficit of fighting behavior. Polish journal of pharmacology. 2004; 56(3):305-312.
40. Ducottet C, Griebel G, Belzung C. Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2003; 27(4):625-631.

How to cite this article: Panditrao A, Pathak P, Pandit Vet al. Preclinical evaluation of antidepressant activity of new monoamine oxidase inhibitors in rodents. Int J Health Sci Res. 2016; 6(9):211-224.

****