ABSTRACT

Aims: To evaluate anti-depressant, anti-anxiety and muscle relaxant activity of hydroalcoholic extract of *Aerva javanica* roots in various experimental animal models.

Study Design: Animal study.

Place and Duration of Study: The study was conducted in Bilwal Medchem and Research Laboratory, Jaipur from July 2021-August 2021.

Methodology: The root powder of *Aerva javanica* was extracted with hydroalcoholic solvent (70% ethanol). The hydroalcoholic extract at three doses 100 mg/kg, 200 mg/kg, and 400 mg/kg was checked for anti-depressant and skeletal muscle relaxant activity in the experimental animal models. To determine the anti-depressant activity tail suspension test, locomotor activity, open field test, and MAO inhibitor assay were done; to determine the anti-anxiety activity hole board test was...
used; and to determine the skeletal muscle relaxant activity rotarod test, grip strength test, and chimney test were done.

Results: In the tail suspension test, the extract at 200 mg/kg and 400 mg/kg significantly reduced the duration of immobility compared to normal control (p<0.0001). The extract at dose 400 mg/kg significantly reduced MAO-A and MAO-B activity compared to the normal control group (p<0.01 and p<0.05, respectively). The extract at 200 mg/kg and 400 mg/kg were able to decrease locomotor activity in actophotometer and increased time spent in centre square in open field test revealing the sedative effect of the extract. In hole board test, the extract at dose 400 mg/kg and 200 mg/kg significantly increased number of head dip count (p<0.0001 and p<0.001) respectively. In the rotarod test, the extract at dose 200 and 400 mg/kg decreased the time spent on the rotating rod (p<0.0001), compared to normal control. Similarly, in the grip strength test the extract at dose 200 and 400 mg/kg decreased the time spend on suspended wire revealing the skeletal muscle relaxant property of the test extract.

Conclusion: Based on the result, it can be concluded that the extract exert anti-depressant, anti-anxiety and skeletal muscle relaxant like activity in the experimental rat which was hypothesized to be attributed to the flavonoids present in the hydroalcoholic root extract of *Aerva javanica*.

Keywords: *Aerva javanica*; Anti-depressant activity; Anti-anxiety activity; Skeletal muscle relaxant activity; Quercetin.

1. INTRODUCTION

Depression accounts for the common and most severe psychiatric disorders across the globe. It is characterized by constant sadness, loss of interest, low energy, sleep disturbances, disruption of daily activities and psychosocial functions. WHO has listed depression as the third largest cause of disease burden since 2008 and it is expected to rank first by 2030 [1].

Conventional medicines to treat depression include tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRIs) and selective reversible inhibitors of monoamine oxidase-A (MAO-A) [2]. As per Cortellis database, by the end of 2019, 828 antidepressants were under development. But, many of these have been discontinued and till now only 292 of these are active and effective. Therefore, there is urgency in the development of new drugs in the field of depression [1].

Major depression is also characterized by physical symptoms which include back pain, limb pain, joint pain along with other physical symptoms. In a study by World Health Organization (WHO), among 1146 patient with depression, 69% reported having somatic symptoms [3]. Skeletal muscle relaxants are prescribed for musculoskeletal conditions such as neck pain, low back pain, tension headache, fibromyalgia, and myofascial pain syndrome [4]. If, in case, the first-line agents fail to relieve pain, skeletal muscle relaxants, benzodiazepines, and opioids may be started based on evidence of temporary additional pain relief [5].

*Aerva javanica* (*A. javanica*) is a perennial herb belonging to the family Amaranthaceae. It is called “bui” locally and “kapok bush” in English. The roots of *A. javanica* has been reported to contain flavonoids such as Quercitin, Aervanone, Chrysin 7-galactoside [6], and the whole plant has been reported to contain isorhamnetin, 3-O-β [4”-p-coumaroyl-a-rhamnosyl (1 →6)] galactoside, Kaempferol, sterol, β-sitosterol, α-amyrin, palmitic acid, linoleic acid, stearic acid, oleic acid, β-amyrin, betulinic acid, phytol, quercitin-3-O-rutinoside, quercetin-3-O-xlyosyl (1→2) rhamnoside, shikimic acid, 10-methoxy-canthin-6-one, and 10-hydroxy-canthin-6-one [7]. It is commonly used in folklore as diuretics, demulcent, in treating kidney stone, pain reliever, anti-acne. It is reported to have anti-inflammatory, hypoglycaemic, anti-parasitic, anti-diabetic, hepatoprotective, anti-microbial, anti-urolithiasis, anti-asthmatic, and anti-fertility activity [8].

To date, no study is reported which highlights the anti-depressant and muscle relaxant activity of *A. javanica*. Therefore, the present study aimed at assessing the anti-depressant as well as skeletal muscle relaxant property of *A. javanica*.

2. MATERIALS AND METHODS

2.1 Plant Material

*A. javanica* was identified and its roots were collected from the local area of Jodhpur, Rajasthan. The plant species were identified and authenticated from the Botany department of Bilwal Medchem and Research Laboratory Pvt.
L eid. Jaipur, Rajasthan. Reference no: BMLPA/2021-16. Roots of A. javanica were cleaned and reduced into small fragments. The fragments were dried at room temperature, under shade till completely dried (15-20 days). After complete drying, the roots were coarsely powdered. The powdered roots of A. javanica were stored in an air-tight container until used.

2.2 Drugs and Chemical

All the solvents used for extraction were laboratory grade and were purchased from LobaChemie Ltd. Fluoxetine (Flunil 20 mg/5 ml suspension) was purchased from Intas Pharmaceutical Ltd., Gujarat, India; Diazepam (Valium 5 mg tablet) was purchased from Abbott Healthcare Pvt. Ltd., Himachal Pradesh, India, Tween 80 used was of pharma grade and was purchased from S.D. Fine Ltd., Mumbai.

2.3 Experimental Animals

Wistar albino female rats (180-200 g) and Swiss mice (20-30 g) were purchased and kept in the animal house of Bilwal Medchem and Research Laboratory Pvt. Ltd., Reengus Industrial area (RIICO), Sikar, Rajasthan. They were fed with a standard diet (standard pellets, Hafed, Rohtak, India) and water ad libitum. The standard housing conditions were maintained viz. 3 animals of same-sex/cage; temperature 22°C (±3°C), humidity 45 -55%, artificial lighting with sequence 12 hours light/12 hours dark. The experimental protocols were as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by Institutional Animals Ethics Committee (IAEC) of Bilwal Medchem and Research Laboratory Pvt. Ltd. (Reg No-2005/PO/RcBT/18/CPCSEA).

2.4 Extraction of Plant Material

The powdered roots of A. Javanica (300 g) were defatted with petroleum ether at 60-80 °C using soxhlet apparatus. The process was continued till a solvent drop from the siphon tube did not leave any greasy spot when evaporated on filter paper. Afterwards, the roots (now weighing 280 gm after defatting) are further extracted with 70% ethanol. The extraction was continued until the solvent in the siphon tube become clear. The extract thus obtained was filtered and collected and evaporated using a rotary evaporator under reduced pressure at 40 °C. The semi-solid extract weighing 72 gm was obtained and was kept in a sealed container in the fridge until further use [9].

2.5 Acute Oral Toxicity Study

The acute oral toxicity of 70% Ethanolic extract of A. javanica was performed as per guideline 423, OECD guideline for testing of chemical, Acute toxic class method, which consists of a single-dose 14-day acute oral toxicity study. Wistar albino female rats (nulliparous, non-pregnant, 8-10 weeks) were used for the study. The rats were fasted overnight, but allowed to have free access to water. A. javanica extract was dissolved in 2% of Tween 80. Afterwards, the rats were administered with a single dose of 2000 mg/kg b.w. p.o (as the text extract is likely to be non-toxic). After the dose administration food but not water was withheld for 4 hours. The animals were observed individually after dosing twice during the first 30 hours, then once in the first 4 hours, then periodically during the first 24 hours, and daily thereafter for 14 days. Observations done include changes in skin and fur, eyes, changes in behavioural pattern, somatomotor activity; respiratory, circulatory, autonomic and central nervous system functions; salivation, diarrhoea, tremors, convulsion, sleep, lethargy [10, 11].

2.6 Experimental Groups

Swiss mice were divided into five groups; Group I served as control and received 1ml/100 gm of 2% Tween 80; Group II served as standard and received diazepam (2 mg/kg, p.o) or Fluoxetine (10 mg/kg, p.o); Group III served as test group 1 and received low dose A. javanica hydroalcoholic extract (100 mg/kg b.w, p.o) denoted as APLD; Group IV served as test group II and received a medium dose of A. javanica hydroalcoholic extract (200 mg/kg b.w) denoted as APMD, and Group V received the high dose of A. javanica hydroalcoholic extract (400 mg/kg b.w) denoted as APHD. The dose of the test was chosen based on an acute oral toxicity study. No toxicity was reported till 2000 mg/kg, b.w, therefore, 1/10 of 2000 mg/kg, b.w i.e. 200 mg/kg, b.w was chosen as medium dose, half of medium-dose i.e. 100 mg/kg, b.w was chosen as low dose, and double of medium-dose i.e. 400 mg/kg, b.w was selected as high dose [12].

2.7 Anti-depressant Activity

2.7.1 Tail suspension test

Various treatments were administered to respective experimental groups of mice for 7
days [13]. 1 hour after the administration of the last treatment the test was started. An adhesive tape was applied at approximately 1 cm above the tip of the tail of mice and the mice were suspended from the edge of the shelf placed at 55 cm above the tabletop. The total duration of the activity was 5 minutes. The mice were considered immobile when they become motionless and hang passively for at least 1 minute [14].

2.7.2 Locomotor activity

Locomotor activity of test extracts was performed using an actophotometer. The actophotometer consisted of a light source, a photocell and a digital counter. The experimental mice of different groups were placed in actophotometer individually and their basal activity score was recorded for 5 minutes. Afterwards, the experimental mice were administered with their respective treatments and after 60 minutes of administration, the activity score was recorded again for 5 minutes [15].

2.7.3 Open-field test

The open field apparatus was kept in a dark, sound-attenuated room and consisted of a white plywood box with dimension 68 x 68 x 45 cm³ with white floor, which was divided into 16 equal compartments using black lines. Various test dose i.e. APLD (100 mg/kg, p.o), APMD (200 mg/kg, p.o) and APHD (400 mg/kg, p.o) were administered once for 7 days to respective experimental groups via the oral route. The standard drug diazepam (2 mg/kg, i.p) was administered once on the 7th day, 30 minutes before the commencement of the test. After 30 minutes of last administration of various treatments, the mice were positioned in the open field test apparatus for 6 minutes and their behavior was recorded. The behavior score included: time spent in the centre square, number of floor lines of the maze crossed, frequency of rearing (number of times the animal stood on its hind limb), and grooming (time duration the animal spent scratching and licking itself while in a stationary position) [2, 16].

2.7.4 MAO assay

Preparation of Mitochondria

Three mice of each group were euthanized. The whole-brain of mice was isolated and kept on ice until further use. Afterwards, the brain was cut into pieces and rinsed with 0.25 M sucrose- 0.1 M Tris-0.02 M EDTA (pH=7.4). The whole content was transferred in a tissue homogenizer and homogenized for 1 minute. Afterwards, the whole content was transferred to a centrifugation machine and centrifuged for 10 minutes at 3500 rpm. The pellets were discarded and the supernatant was then centrifuged at 12000 rpm for 20 minutes. The precipitate was washed with 10 ml of sucrose-tris EDTA and resuspended in 5 ml of the medium. The resulting mitochondrial suspensions were frozen for two days at -18°C. To the defrosted mitochondrial suspensions, an additional 5 ml of sucrose-tris-EDTA medium was added and the mixture was homogenized and centrifuged at 12000 rpm for 20 minutes. The pellets were suspended in 50 mM of phosphate buffer, pH 7.4. This preparation was then used for MAO assay [17].

The final protein concentration in the above mitochondrial mixture was estimated by the Lowry method. The MAO assay mixture consisted of 4 mM 5-HT and 2 mM beta-phenylalanine as a specific substrate for MAO-A and MAO-B respectively, 250 μl of mitochondrial fraction, and 100 mM sodium phosphate buffer (pH 7.4) to make a final volume of 1 ml. The reaction mixture was kept at 37°C for 20 minutes for the reaction to proceed and was stopped by adding 200 μl of 1 M HCl. The reaction product was extracted with 5 ml of butyl acetate for MAO-A assay and cyclohexane for MAO-B assay. The organic phase was read at 280 nm for MAO-A assay and cyclohexane for MAO-B assay. Blank was prepared by adding 200 μl of 1 M HCl before reaction [18].

2.8 Anti-anxiety Activity

2.8.1 Hole board test

The hole board test evaluate the exploratory behavior of rodent when exposed to new environment, which is characterized by head dip in the hole board. It is as apparatus with a with dimension 40*40 cm with twenty equidistant holes of diameter 4 cm. The experimental mice were administered with their respective treatment. After 30 min of drug administration, the mice were placed at the center of the apparatus and were allowed to explore the arena. The number of head dip made by the mice of each group was counted for the duration of 5 minutes [15].
2.9 Muscle Relaxant Activity

2.9.1 Rota rod test

Rotarod apparatus consisted of a bar of 7 cm diameter placed at a distance of 24 cm above the floor of the apparatus and was set at a speed of 25 rpm. Before conducting the test, the mice were trained for three consecutive trials. On the next day, the mice were placed individually on the rotarod (set at 25 rpm) and the fall-off time was recorded for 5 minutes. Afterwards, mice of various groups received their respective treatments. After 30 minutes of administration of various treatments, the mice were again placed on the rotating rod and fall-off time was recorded [19].

2.9.2 Grip strength test

The experimental mice were placed on the horizontal thin thread wire (30 cm length) suspended about 50 cm into the air. As a normal behavior, the mice will immediately grasp it with their forepaws. The mouse was exposed to hang onto the suspended wire threat with its forelimb. Normal mice would be able to catch the thread with the hind limbs and would tend to climb up within 5 seconds will be included in the study [2]. The length of time during which the mouse was able to hold the suspended wire was recorded and this latency of the grip loss served as an indirect measure of grip strength. The mice were administered with respective treatment and after 60 minutes of administration of treatments, each mouse was kept on the wire and the time at which the animal falls off from the wire was recorded [20].

2.9.3 Chimney test

For this test, a pyrex-glass cylinder of 30 cm length was used. Firstly, a mark was made at 20 cm from the base of the cylinder. Afterwards, the pyrex-glass cylinder was held in a horizontal position and at the end of the cylinder, near the mark, a mouse was introduced with the head forward. As the mouse reached the other end of the cylinder, the cylinder was moved to a vertical position. As the position of the cylinder was changed from horizontal to vertical, the mouse immediately tries to climb backwards. The time required by the mouse to climb back at the top of the cylinder was recorded for each experimental group of mice [2].

2.10 Statistical Analysis

The Statistical tool used was Graph pad prism version 9. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett’s test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; **p< 0.001; *p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.

3. RESULTS AND DISCUSSION

3.1 Acute Oral Toxicity

No mortality or moribund status was observed in experimental rat with a single dose of 2000 mg/kg, hence, the test extract was considered safe in rats and the dose of 100 mg/kg, b.w; 200 mg/kg, b.w; and 400 mg/kg, b.w were selected for further pharmacological evaluation.

3.2 Anti-depressant Activity

3.2.1 Tail suspension test

As shown in Fig. 1, APMD 200 mg/kg and APHD 400 mg/kg significantly reduced the duration of immobility at p<0.0001 compared to the normal control group. However, the effect of APLD 100 mg/kg in lowering the duration of immobility was not significant compared to the normal control group.

3.2.2 Locomotor activity

As shown in Table 1 administration of Diazepam, APLD, APMD, and APHD significantly increased the time spent in the central square at p<0.0001. Administration of Diazepam depicted a significant decrease in rearing time in experimental mice, whereas none of the test treatments viz APLD, APMD, and APHD decreased the rearing time compared to control. A significant decrease in grooming time was noted in Diazepam and APHD at p<0.0001; APMD at p<0.001; and APLD at p<0.05.
Fig. 1. Effect of various treatments on duration of immobility (sec) in Tail suspension test. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett’s test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; ***p< 0.001; **p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.

Table 1. Effect of various treatments on locomotor activity of experimental mice using Actophotometer

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment groups</th>
<th>Mean Score in 5 mins before treatment administration</th>
<th>Mean Score in 5 mins after treatment administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>289.166±4.150</td>
<td>284.333 ±6.047</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam (2 mg/kg, i.p)</td>
<td>282.5 ± 3.373 ns</td>
<td>121.5 ± 4.372</td>
</tr>
<tr>
<td>3</td>
<td>APLD 100 mg/kg, p.o</td>
<td>282.166 ±2.700 ns</td>
<td>255 ± 10.201</td>
</tr>
<tr>
<td>4</td>
<td>APMD 200 mg/kg, p.o</td>
<td>287.666 ±3.929 ns</td>
<td>204.5 ± 3.676</td>
</tr>
<tr>
<td>5</td>
<td>APHD 400 mg/kg, p.o</td>
<td>284.833 ±3.239 ns</td>
<td>164.333 ±3.451</td>
</tr>
</tbody>
</table>

Table 2. Effect of various treatments on behavior score of experimental mice in open-field test method

<table>
<thead>
<tr>
<th>S. N</th>
<th>Treatment group</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time spent in the centre square (secs)</td>
<td>Rearing time (secs)</td>
</tr>
<tr>
<td>1</td>
<td>Normal control</td>
<td>8.89±0.735</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam (2 mg/kg, i.p)</td>
<td>118.92 ± 1.12 ns</td>
</tr>
<tr>
<td>3</td>
<td>APLD (100 mg/kg, p.o)</td>
<td>45.87 ± 2.439 ns</td>
</tr>
<tr>
<td>4</td>
<td>APMD (200 mg/kg, p.o)</td>
<td>56.436 ± 2.55 ns</td>
</tr>
<tr>
<td>5</td>
<td>APHD (400 mg/kg, p.o)</td>
<td>67.506 ± 1.998</td>
</tr>
</tbody>
</table>
3.2.4 MAO assay

As shown in Table 3, MAO-A and MAO-B activity in the normal control group was found to be 24.63±0.859 and 24.44±1.187 respectively. There was a significant decrease in MAO-A activity in Fluoxetine 10 mg/kg group and APHD 400 mg/kg at p<0.01 and per cent inhibition of MAO-A activity by Fluoxetine 10 mg/kg group and APHD 400 mg/kg was 31.34 and 27.96 respectively. Inhibition of MAO-A activity by APLD 100 mg/kg and APMD 200 mg/kg was not significant. In the case of MAO-B activity, a significant decrease was noted by Fluoxetine 10 mg/kg and APHD 400 mg/kg at p<0.01 and p<0.05 respectively and the per cent inhibition of MAO-B activity by both the group was found to be 40.87 and 32.39 respectively. APLD 100 mg/kg and APMD 200 mg/kg does depict any significant decrease in MAO-B activity.

3.3 Anti-anxiety Activity

3.3.1 Hole board test

Diazepam and APHD 400 mg/kg significantly increased head dip compared to the mice of normal group at p<0.0001. The anxiolytic effect of APMD was also significant at p<0.001 compared to normal control. However, APLD does not increase head dip to significant level and does not showed anxiolytic activity. The result is presented in Fig. 2.

Table 3. Effect of various treatments on MAO activity in mouse brain

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment groups</th>
<th>MAO activity nmol/mg of protein</th>
<th>Percent inhibition of MAO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAO-A</td>
<td>MAO-B</td>
</tr>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>23.63±0.859</td>
<td>24.44±1.187</td>
</tr>
<tr>
<td>2</td>
<td>Fluoxetine (10 mg/kg)</td>
<td>16.23±0.649</td>
<td>14.45±0.763</td>
</tr>
<tr>
<td>3</td>
<td>APLD 100 mg/kg, p.o</td>
<td>23.71±1.262 ns</td>
<td>25.08±1.337 ns</td>
</tr>
<tr>
<td>4</td>
<td>APMD 200 mg/kg, p.o</td>
<td>20.72±0.405 ns</td>
<td>22.55±1.348 ns</td>
</tr>
<tr>
<td>5</td>
<td>APHD 400 mg/kg, p.o</td>
<td>17.02±0.757 ns</td>
<td>16.52±0.777 ns</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of various treatment on head dip count in hole board test. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett’s test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; ***p< 0.001; **p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.
3.4 Muscle Relaxant Activity

3.4.1 Rota rod test

As seen from Fig. 3 experimental mice of APMD 200 mg/kg and APHD 400 mg/kg significantly reduced the time spent on rotating rod after their treatment administration at p<0.0001, when compared to normal control, and the effect of the test extract was dose-dependent. The experimental mice in the Diazepam group significantly reduced the time spent on rotating rod at p<0.0001 compared to control. However, the effect of APLD 100 mg/kg was in reducing the time spent on rotating rod was not significant when compared to control.

3.4.2 Grip strength test

As shown in Fig. 4 experimental mice treated with APLD 100 mg/kg were not quickly fallen from the suspended wire, depicting its inability to relax the muscle. While treatment with Diazepam, APMD 200 mg/kg and APHD 400 mg/kg the time spent on the suspended wire were significantly reduced as compared to normal control depicting its skeletal muscle relaxant activity.

3.4.3 Chimney test

As shown in Fig. 4, APLD 100 mg/kg took less time to leave the cylinder depicting normal muscle coordination. However, treatment with Diazepam, APMD 200 mg/kg and APHD 400 mg/kg displayed a significant increase in time to leave the cylinder compared to normal control at p<0.0001 and it depicts that Diazepam, APMD and APHD acted as a potent skeletal muscle relaxant.

The present study dealt with the possible anti-depressant, anti-anxiety and skeletal muscle relaxant activity of A.javanica root extract. The idea of selecting roots of this particular plant for study is, firstly, the roots are rich in flavonoids such as Quercitin, Chrysin-7-galactoside, and Aervanone [6] (as per literature survey) which are reported to have evidence based anti-depressant and skeletal muscle relaxant activity; Secondly, no such evaluation of roots or any part of A. javanica has been reported for anti-depressant, anti-anxiety skeletal muscle relaxant activity previously.

![Fig. 3. Effect of various treatments on average fall off time of experimental mice using Rota rod apparatus. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett's test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; ***p< 0.001; **p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.](image-url)
Fig. 4. Effect of various treatments on average fall off time of experimental mice using Grip strength test. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett’s test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; ***p< 0.001; **p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.

Fig. 5. Effect of various treatments on time taken by experimental mice to leave the cylinder using Chimney test. All the values are expressed as Mean ± SEM and are analysed using One way ANOVA followed by Dunnett’s test of multiple comparisons. The total number of animal per group is 6 (n=6), and the criterion of statistical significance was **** p<0.0001; ***p< 0.001; **p<0.01; *p<0.05 when the mean of the normal control group was compared with the means of other treatment groups. NS denotes not significant.
For evaluation of anti-depressant activity tail suspension test, locomotor activity, open field test, and MAO inhibition assay was done. Tail suspension test is a common behavioural model utilized for the evaluation of the antidepressant activity of any experimental drug. This test involves the suspension of the mice via their tail by some distance from the floor and the behaviour of the mouse to counter this aversive situation is noted during the time, however, due to unsuccessful attempt, the mouse experience despair and become immobile. The extent of immobility is considered to be a depiction of the depressive-like condition of experimental mice and is thought to be significantly reduced by antidepressant drugs. Unlike the forced swimming test, the tail suspension test is the best-approved test for the evaluation of anti-depressant like effects of drugs with the additional benefit of not causing hypothermia as in the forced swimming test. Moreover, it is also used to evaluate the effects of hereditary, ecological, and neurobiological controls. Our test extracts at dose 200 and 400 mg/kg were significantly able to reduce the duration of immobility and hence, can be said to have anti-depressant like property [21].

In case of locomotor activity, it was observed from the result that the extract tend to lower the locomotion compared to the normal control group which depicts the sedative effect of the extract. There are many sedative anti-depressant drugs such as TCA e.g. amitryptiline, doxepin, nortriptiline, nortriptiline, mianserin; serotonin receptor 5-HT2 antagonists (SARIs) e.g. trazodone and nefazodone. These sedative antidepressant are known to act via two mechanisms i.e. by blocking histamine H1 receptors and serotoninergic 5-HT2A receptors and antagonism of H1 receptor induces strong sedation [22]. Therefore, it may be possible that the A. javanica test extract may have some H1 or 5-HT2 receptor blocking property responsible for its sedation.

Assessments of stress-related anxiety in rodents are evaluated using several behavioural tests and the open field test is one of those [23]. It is widely used to evaluate the emotionality and locomotor performance in rodents [24]. Additionally, it is also used to evaluate the sedative, stimulant, or toxic effects of the compounds. Therefore, this test has several uses and is included in most rodent behaviour analysis [25]. In our test we evaluated three parameters related to open-field test viz. time spent in the centre square of the field, rearing time, and grooming time. Increased time spent in the central square depicts reduced locomotion. The Diazepam administered mice depicted more time spent in the centre square and less locomotion, and administration of APLD 100 mg/kg, APMD 200 mg/kg, and APHD 400 mg/kg also increased the time spent in the centre square compared to the normal control group. Rearing is the duration in which animals spent time in an erect posture to explore the surrounding during the test period [26]. Experimental mice administered with APLD, APMD, and APHD showed normal rearing behaviour whereas the mice administered with Diazepam showed decreased rearing compared to mice of the normal control group. Change in the rodent's depression-like states has been reported to influence grooming. However, the effects of depression on grooming are somewhat delayed and less obvious. As grooming only present one domain. Hence, other behaviour parameters are required for in-depth ethological evaluation. Diazepam has been reported to normalize grooming pattern by reducing the incorrect transition percentage and interrupted bout percentage [27]. APHD also was able to reduce grooming and its effect was comparable to Diazepam.

MAO-A inhibition is one of the established mechanisms of anti-depressants. Reversible as well as irreversible inhibitors of MAO-A are used as anti-depressants. The function of MAO is the metabolism of xenobiotics, neurotransmitters, and endogenous amines such as serotonin, dopamine, noradrenaline, tyramine, tryptamine, etc. MAO occurs in two isoforms MAO-A and MAO-B. MAO-A is involved in depression and psychiatric condition whereas, MAO-B is involved in neurodegenerative diseases. MAO-A inhibitors act as potent anti-depressants whereas MAO-B inhibitors are thought to be neuroprotectants. Additionally, MAO induced oxidation of biogenic amines and neurotransmitters leading to the production of hydrogen peroxide (H₂O₂), reactive oxygen species which elevate the risk of oxidative injury. Therefore, MAO inhibition may be responsible for imparting protection against oxidative stress. It is reported that plant extracts may inhibit MAO leading to the above-mentioned effects [28]. In our study, A. javanica plant extract at dose 400 mg/kg inhibited MAO-A as well as MAO-B which strongly suggests the role of the extract in exerting anti-depressant activity. The effect of inhibition of MAO-A was more prominent than MAO-B.
For anti-anxiety activity, hole board method was used. The presence of flavonoid [6] may be responsible for its anti-anxiety activity.

For evaluation of muscle relaxant activity rotaorad test, grip strength test, chimney test were used. The Rotaorad test is used to assess the activity of drugs interfering with motor coordination and is evaluated by testing the ability of the rodents to stay on a revolving rod. The endpoint of the test is when the dose impairs the ability of 50% of rodents to stay on the revolving rod [29].

Grip strength test is used for assessment of neuromuscular function and muscular strength in rodents known to be influenced by sedative drugs, muscle relaxant compounds and toxic agents [30]. As revealed by rotaorad, grip strength, and chimney test, significant muscle relaxation was shown by diazepam, APME 200 mg/kg, and APHD 400 mg/kg. The mechanism of relaxation may be attributed to the interaction of the test extract with the benzodiazepine site on the GABAA BZD chloride ion channel receptor complex system. GABAA BZD chloride ion channel receptor complex is known to mediate the central muscle relaxation action of drugs. It may be possible that muscle relaxation [31].

This can further be supported by studies that have demonstrated the increased levels of GABA and GABA release following high dose acute administration of tricyclic antidepressants, MAOIs. Recently, phenelzine, an MAO inhibitor was found to increase GABA levels in the brain due to GABA-T inhibition. The elevated level of GABA following the administration of SSRI has been implicated in human studies. TCAs such as amitriptyline, imipramine, and desipramine were reported to increase GABA level, GABAA receptor subunit expression and inhibit GABA transporters in the brain. SSRIs such as Fluoxetine is known to increase GABAA receptor activity. Fluvoxamine and fluoxetine were reported to change the neurotransmitter steroid concentration which in turn facilitates GABA function [32]. The biochemical causes of depression involve the monoamine neurotransmitter metabolic disorder that participates in noradrenaline (NE), serotonin (5-HT), and dopamine (DA) signalling and MAO activity. Additionally, there is a strong association of Brain-derived neurotrophic factors (BDNF) and gamma-aminobutyric acid (GABA) with depressive disorders [33]. The roots of A. javanica has been reported to contain flavonoids such as Quercitin, Aervanone, Chrysin 7-galactoside [6].

Quercetin is reported to enhance the availability of 5-HT and norepinephrine in the synaptic cleft that is known to be dysregulated in depression. Quercetin along with other flavonoids have been reported to subside the depressive symptoms in in-vitro experimental models and the action was attributed to inhibition of MAOs, alteration of oxidative defences and modulation of the GABAA system [34]. Additionally, it might act as a ligand for the BZD binding site of y-aminobutyric acid type A (GABAA) receptor which is confirmed by many behavioural animal models of anxiety, sedation, and convulsions. It is also reported to improve serotonergic functions [35].

Several studies have demonstrated the anti-depressant effect of flavonoids fraction present in the plant extract. For instance, a crude extract of Hypericum perforatum composed of flavonoids such as querciton, isoquercitin, miquelianin, hyperoside, and astilbin and its showed an anti-depressant like effect in mice. Similarly, methanolic extract of Brysonima crassifolia consisted of quercetin, rutin, hesperidin, quercetin 3-O-xylloside, hesperidin and the extract exerted an anti-depressant like effect in forced swim test in mice. Isolation of flavonoids from methanolic extract of Cayratia japonica revealed the presence of quercitin, pigenin, luteolin, taxifolin and the extract showed anti-depressant like activity which was attributed to MAO inhibition. Quercetin is known to inhibit MAO-A and MAO-B efficiently and the inhibition of more prominent for MAO-A [33].

In a study, skeletal muscle relaxant activity and locomotor activity of quercetin and chrysine were reported in an experimental animal model. And, chrysine and quercetin induced skeletal muscle relaxation and reduced locomotion. However, the effect of chrysine was statistically significant and that of quercetin was not significant [36]. Flavonoids in low concentration have been reported to modulate GABAA receptor in both flumazenil-sensitive or flumazenil insensitive manner i.e. it can modulate GABAA receptor independently as well as through the classical benzodiazepine-binding site [37]. The effect of Chrysine was studied in rat and its effect was linked to activation of the GABA receptor unit [38].
4. CONCLUSION

Based on the result we hypothesize in our study that the roots of *A. javanica* are rich in flavonoids viz Quercitin, Aervanone and Chrysin 7-galactoside which may be responsible for anti-depressant, anti-anxiety and skeletal muscle relaxant action via inhibition of MAO-A and MAO-B, enhancement of 5-HT and norepinephrine availability in the synaptic cleft, modulation of GABAA receptor, and alleviating the oxidative stress.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

The experimental protocols were as per committee for the purpose of control and supervision of experiments on animals (CPCSEA) and were approved by institutional animals ethics committee (IAEC) of Bilwal Medchem and Research Laboratory pvt. Ltd. (reg no- 2005/PO/RcBT/18/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/79708