Role of traditionally used Indian medicinal plants in animal models of depression with reference to flavonoid-rich ethanol extract of *Abrus precatorius* and *Neolmarckia cadamba* leaves

Pritam Gokuldas Bhore  
Department of pharmacy, Mandsaur University, Mandsaur, 458001, Madhya Pradesh, India  
Corresponding author email: pgb.ccopr@gmail.com

Dr. Swapnil Goyal  
Department of pharmacy, Mandsaur University, Mandsaur, 458001, Madhya Pradesh, India

**Abstract**---Flavonoid is an active phytoconstituent that was identified from ethanol extract of *Abrus precatorius* (ApEe) and *Neolmarckia cadamba* (NcEe) leaves. In the present study, flavonoid-rich ethanol extract of both plants was studied for its antidepressant activity in stress induced depression. The phytochemical study reveals the presence of flavonoids, as flavonoids shows significant effects to improve the mood. It plays an important function in stress-induced depression; hence in this study includes pharmacological evaluation for the antidepressant effect of ethanol extract of both plants which was examined separately. The antidepressant activity of the prepared extract was estimated by despair animal models. In the present experiment, we selected forced swim test in the rat (rFST), mice tail suspension test (mTST) and locomotor activity was also examined in the open field test. A Comparative profile of the test formulation of ethanol extracts of *Abrus Precatorius* (ApEe) and *Neolmarckia Cadamba* (NcEe) was assessed for effect on immobility time in rFST and mTST at dosages 100 mg/kg and 200mg/kg. ApEe shows significant \( (p<0.001, 77.17\pm1.537) \) reduction of immobility duration time in rFST at 200 mg/kg dose, however in mTST it was significant \( (p<0.003, 98.67\pm16.77) \) with 100 mg/kg and more significant \( (p<0.0138, 78\pm2.716) \) at 200mg/kg. NcEe significantly \( (p<0.001, 137\pm3.347) \) reduce immobility duration in rFST at 100mg/kg and significantly \( (p<0.001, 95.17\pm4.47) \) reduce immobility with increasing dose 200 mg/kg. However NcEe significantly \( (p<0.001, 80.50\pm2.77) \)
reduced immobility in mTST at a high dose 200 mg/kg. During neuropharmacological evaluation, both extracts show a dose-dependent significant reduction of immobility time in rFST and mTST at dosages 100 mg/kg and 200mg/kg respectively, hence the ethanol extract of both plants possess antidepressant activity in stress-induced depression.

**Keywords**— *Abrus precatorius*, *Neolmarckia cadamba*, Flavonoid, rFST, mTST, Antidepressant.

**Introduction**

Depression is mental illness which characterized by loss of interest and inability to do daily activities. Depression usually occurred at a younger age due to the social and financial burden of individuals. As per World Health Organization (WHO), in 2015 about 4.4% peoples of global population suffering from depression. The neurotransmitters like serotonin, acetylcholine, norepinephrine, etc. play active role in the regulation of the functions of a nervous system. Monoamine oxidase (MAO) is a mitochondrial enzyme found in nerve cell. This MAO is acting as a safety valve because it deaminates and inactivates neurotransmitters like norepinephrine, dopamine, and serotonin, and causes neurotransmitter deficiency. Neurotransmitter deficiency leads the major depression. Major depression is a complex neuropsychiatric disorder that refers to pathological changes in mood state and is characterized by symptoms like sad mood, psychomotor retardation, and loss of interest. Depression is a major disease of public health which is prevalence, suffering dysfunction, economic burden, and morbidity. The Global Burden of Disease stated that unipolar depression about 1.9% for me and 3.2% for women. They also estimate depression will be the most leading cause of disability of life and ischemic heart disorder. Various studies stated that depression in the community sample is varied from 1.7 - 74 per thousand population. WHO estimated depression become the second largest illness in the world and declared that depression is the second cause of heart disease by 2020. Today there are several synthetic antidepressant drugs are available but these drugs have restrictions and limitations in clinical use due to adverse effects, hence to avoid or minimize the severe adverse effect and toxicity of synthetic drugs it is necessity to find out alternative antidepressants which obtain from natural sources like herbal medicine which are used traditionally in Ayurveda. Ayurveda is a traditional medicinal system of India which consists of various types of medicinal herbs to prevent and treat various diseases or disorders. *Atharvaveda, Charak –Samhita*, and *Sushrut- Sanhita* are the main classic of *Ayurveda* which gives us detailed information and description of more than 700 herbs. Today several studies have investigated and reported potential antidepressant activity of the natural chemical compound obtained from medicinal herbs. It is necessary to develop the new and potential antidepressant from ayurvedic medicinal herb whose neuropharmacological potential will assessed in a variety of experimental rodent animals. In Sanskrit, *Abrus precatorius* is known as Gunja. In English, it is called jequirity bean (rosary pea). This plant consists of flowers and it belongs to the family Fabaceae. The Roots, leaves, seeds of this plant were majorly used as
Ayurveda medicine. Roots of this plant contain phytoconstituents like abresine, abrol, precool, and precasine. Seeds are rich in abrine, arbusgenic acid, choline, calcium, and anthocyanins. Isoflavonoids and quinones are present in leaves. The White variety of these plant leaves is traditionally used to prepare oil which claims to be aphrodisiac, used in eye disease, use to cure leucoderma, wounds, skin disease, asthma, cancer, as sedative. Tea from its leaves is uses for fever, cough, and cold. A mixture of leaves and honey is used to treat swelling. In Ayurveda, this plant uses to promote hair growth. Seeds of this plant consist of flavonoids, alkaloids, steroids. Paste obtained from seeds applied locally in sciatica, shoulder joint stiffness, and other nervous diseases. In white leprosy, seed paste is applied as a stimulant dressing. Roots of this plant are used in heart disease, kidney disease, cancer, and as sedative. Neolmarckia cadamba is a tropical plant from the family Rubiaceae. In Ayurveda, it is known as kadamb or kadam. In the Indian endogenous medicinal system-Ayurveda, various parts of this plant have been used to treat many diseases. The major active phytochemicals of this plant are alkaloids, carbohydrates, protein, steroids, gum, saponin, triterpenoids, tannins, and flavonoids. Paste obtained from Neolmarckia cadamba leaves apply to treat wounds and their pain and swelling. Leaves extract of cadamba is used to cure diabetes and used as a mouth gargle. The bark of this plant is traditionally used to treat fever, blood-related diseases, mouth ulcers, and inflammation. Roots are useful to cure urinary tract infection; renal calculi and muscular pain. Paste of flower applies to treat black spot and pimples. The Arial part has beneficial properties in diarrhea and irritable bowel syndrome. Abrus precatorius and Neolamarckia cadamba are traditionally used medicinal plants as per Ayurveda. These plants were used traditionally to treat various diseases. The ethanol extract of both plants shows the presence of flavonoids after phytochemical testing. The flavonoids have shown effect on the state of mood improvement, a nervous system disorder which plays important role in stress induces depression. Hence this study was undertaken to evaluate the effect of flavonoid-rich ethanol extract of Abrus precatorius and Neolamarckia cadamba in despair animal models of experimentally induced acute depression. When results are significant, the investigated product will be subjected to a structural elucidation of flavonoid and its derivatives and herbal product will be helpful in the management of depression. The extracts of Abrus precatorius and Neolamarckia cadamba may have antidepressant activity, which shows their potential to be used as an herbal antidepressant drug.

Material and Methods

Collection, Authentication, and Processing of plant material

Fresh leave of Abrus precatorius was collected in November-2020 from the geographical region of Ahmednagar Districts (Maharashtra, India). Fresh leave of Neolmaeckia cadamba was collected in December-2020 from the geographical region of Pune Districts of Maharashtra state. Both plants were authenticated from the Botanical Survey of India, Pune. Leaves of plants were separated and cleaned by pure water. Collected leaves were subjected to shade dried for two weeks at room temperature.
Preparation of extract

The powder material of *Abrus precatorious* and *Neolmaeckia cadamba* was employed to batch extraction in Soxhlet’s apparatus. The solvents used were Petroleum ether, Chloroform, Ethanol, and Distilled water. Prepared extracts stored in well-closed container\textsuperscript{12, 13}.

Preliminary phytochemical screening

Prepared ethanol extract of *Abrus precatorious* and *Neolmaeckia cadamba* was subjected for preliminary phytochemical investigation by using the following qualitative chemical tests\textsuperscript{14, 15}.

Test for carbohydrates

Molisch’s test: To 3-4 ml of extract add a few drops of α-naphthol, shake it well, and to it mix concentrated H\textsubscript{2}SO\textsubscript{4}. At the junction of liquids observe a violet color ring.

Test for alkaloids

Dragendorff’s test: To 3-5 ml filtrate mix with a Dragendorff’s reagent, observe orange-brown precipitate.

Mayer’s test: Filtrate adds with Mayer’s reagent till formation of precipitate.

Test for glycosides

Baljit’s test: Extract with sodium picrate shows orange color.

Raymond’s test: Test solution hot with methanol alkali solution and observe for violet coloration.

Shinoda test for flavonoids

In dry powder of extract add 3 ml of ethanol, to it conc. HCL, 0.5 gm magnesium turnings. Observe orange, pink and purple colors for flavonol, di-hydro derivative, and xanthene.

Solubility test for fats and oils

Oils are insoluble in ether, benzene, and chloroform but insoluble in 90%ethanol and water.

Test for phenolic compound and tannins

Add following reagents to 3-4ml of extracts:

Lead acetate solution: deep blue color appears.

Bromine water: Decolouration of bromine water.

Acetic acid solution: Red color appears.

Dilute iodine solution: Transient red color appear.

Pharmacological evaluation

Experimental animals

Healthy Albino Wister rat (160-180gm), Albino Swiss mice (18-30 gm.) were used for the experiment under controlled laboratory conditions as well as exposed to 12
hours light cycle. IAEC approval obtained from IAEC of B. R. Nahata College of Pharmacy, Mandsaur, M.P as project proposal no. IAEC/BRNCOP/2020/004.

**Acute toxicity study**

Acute toxicity study for *Abrus precatorius* and *Neolmarckia cadamba* leaves extract was done in mice as per OECD guideline 420. Fixed-dose method was used for dose determination. For the forced swim test in rats, one day before the experiment, the rats were brought to the laboratory. During the experiment, rats were individually forced to swim inside the acrylic cylinder which contains water at 25°C temperature. The duration of immobility was observed and recorded for the individual rats. Ethanol extracts obtained from leaves of *Abrus precatorius* and *Neolmarckia cadamba* were used for acute study. To perform the forced swim test, rats of either sex weigh about 160-180 gm (n=6). The first group (control/vehicle) was administered saline solution (0.2 ml/animal), the second group was administered Imipramine (15 mg/kg), the third group was administered test extract of low dose (100 mg/kg), and the fourth group was administered test extract of high dose (200 mg/kg). The oral route of drug administration was employed for the experiment. Rat forced swim test consists of two parts; part one is the pretest, and part two is the main. During the pretest, rats force for swim for fifteen minutes at constant temperature in water, after fifteen minutes of the test session, animals were dried by using a clean cloth and placed into a clean cage. After 24 hours of pretest main test was conducted. During the main test session, the last six minutes are considered observing and measuring immobility parameters in rats. Immobility defines as floating in the water without struggling and trying to climb movement to escape from water. For the mice tail suspension test, it is also known as the dry test. This test is used to evaluate antidepressant activity. In this test, the animal was hung to the horizontal stand and maintains 50 cm distance from the floor. Ethanol extract of *Abrus precatorius* and *Neolmarckia cadamba* tested to an evaluation of antidepressant activity by using tail suspension test in mice of either sex and weigh about 20-27 gm. All test animals divide into four groups (n=6). Oral route of drug administration was preferred during the experiment. The control group received saline solution (0.2 ml/animal), the second group administered imipramine (15 mg/kg), and the third group administered ethanol test extract at a low dose (100 mg/kg). The fourth group administered ethanol test extracts (200 mg/kg). After one hour of administration mice tail suspension test was performed as per procedure and record duration of immobility (in seconds) during the last four minutes of a test session. The Immobility parameter was considered when mice passively hang without any movement and motions.
Assessment of locomotor activity

Drugs which act on the central nervous system affect locomotor activities in humans as well as animals. Locomotor activity checks to determine wakefulness of mental activity; hence actophotometer test was performed to estimate the effect of test extract on locomotor activity. During this activity, animals were treated with test extract at 100mg/kg and 200mg/kg dose and compared activity with control (saline) and standard (imipramine) treated groups of animals. During the experiment individual animals were placed into actophotometer for 10 minutes. Locomotor activity score recorded as on the digital display of actophotometer21, 22.

Statistical analysis

ANOVA (One-way Analysis of Variance) followed by Dunnet’s test was used to obtained statistical data. The level of significance was fixed at p < 0.05. All data expressed as mean ± Standard Error Mean (SEM).

Results

Phytochemical screening

The phytochemical screening of ethanol extract of Abrus Precatorius (ApEe) and Neolmarckia cadamba (NcEe) leaves shows the presence of flavonoid, glycoside, alkaloids, carbohydrates, tannins, fats, and oil.

Acute toxicity study

ApEe and NcEe extracts were found to be safe in mice up to the 2000mg/kg dose level. Extracts did not show any toxicity and hence both extract use for further neuroprotective and behavioral screening.

Effect of ApEe on the duration of immobility in rat force swim test

After 24 hours of the preliminary test, immobility parameter was recorded for test extract at dose 100mg/kg and 200mg/kg. In a low dose of ApEe (100mg/kg) treated animals there is no significant reduction in immobility time as compared to the control/saline group. Imipramine (Standard) and high dose of ApEe (200mg/kg) treated animal shows more significant (p<0.001) decrease in duration of immobility when compared to the control group. A statistically significant reduction in the duration of immobility time was observed and recorded in low and high doses of ethanol extract of Abrus precatorius treated group of animals. The result shows a dose-dependent effect. In the experiment, high dose (200mg/kg) of ethanol extract shows a significant effect as compared to standard treated (imipramine (15mg/kg) animal. Imipramine shows the superior effect as compared to 100mg/kg (low dose) of ethanol extract, hence a high dose (200mg/kg) of the extract can be considered as an antidepressant dose. The immobility time parameter is explained in table -1.
Table 1
Effect of ApEe-1 and ApEe-2 in rFST. Values expressed as mean ± SEM, n=6, p-values, ns-non significant (ApEe-1), p<0.001 (ApEe-2) when compared with control treated group

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Mean Duration of Immobility Time(sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>192.3±2.076</td>
</tr>
<tr>
<td>2</td>
<td>Imipramine(15mg/kg)</td>
<td>86.33±2.305***</td>
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<tr>
<td>3</td>
<td>ApEe-1 (100mg/kg)</td>
<td>132.2±8.968ns</td>
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<tr>
<td>4</td>
<td>ApEe-2 (200mg/kg)</td>
<td>77.17±1.537***</td>
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</table>

Effect of ApEe on the duration of immobility time in mice tail suspension test

Acute effect of 100mg/kg 200mg/kg of extract is summarized in table 1. Immobility time was observed for the last 4 minutes of a test session. Both doses significantly reduced immobility time (p<0.013, p<0.003). The effect of high dose (200mg/kg) was nearly equivalent as compared to imipramine (Standard treated animal). Effect of low dose (100mg/kg) also shows a significant reduction in duration of immobility in mice but minimum as compared to high dose. The decrease in immobility time parameter is explained in table 2.

Table 2
Effect of ApEe-1 (100mg/kg) and ApEe-2 (200mg/kg) on duration of immobility in mice tail suspension test. Values expressed as mean ± SEM, n=6, p-values, p<0.013 (ApEe-1), p<0.003 (ApEe-2) when compared with control treated group

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Mean Duration of Immobility Time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>137.2±2.750</td>
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<tr>
<td>2</td>
<td>Imipramine(15mg/kg)</td>
<td>76.17±2.056***</td>
</tr>
<tr>
<td>3</td>
<td>ApEe-1 (100mg/kg)</td>
<td>98.67±16.77*</td>
</tr>
<tr>
<td>4</td>
<td>ApEe-2 (200mg/kg)</td>
<td>78.33±2.716***</td>
</tr>
</tbody>
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Assessments of locomotor activity in the rat after administration of ApEe

Score of locomotor activity observed and record it after a dose administration by using actophotometer. Locomotor activity was assessed for ten minutes test session during experiment test animals receiving vehicle (saline solution), imipramine (15mg/kg), and ethanol extract (200mg/kg). As per the locomotor activity score, there is no significant effect on the animal was observed as compared to a vehicle-treated animal. Results of the activity are shown in table 3.
Table 3
Effect of ApEe-1 (100mg/kg) and ApEe-2 (200mg/kg) on locomotor activity in the rat. Mean ± SEM, n=6, ns= Non-significant

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Locomotor Activity Score</th>
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<tr>
<td>1</td>
<td>Control</td>
<td>351.3±6.505</td>
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<tr>
<td>2</td>
<td>Imipramine (15mg/kg)</td>
<td>336.2±7.181ns</td>
</tr>
<tr>
<td>3</td>
<td>ApEe-1 (100mg/kg)</td>
<td>324.3±5.445ns</td>
</tr>
<tr>
<td>4</td>
<td>ApEe-2 (200mg/kg)</td>
<td>325.3±6.712ns</td>
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</table>

Effect of NcEe on the duration of immobility in rat force swim Test

A statistically significant decrease in immobility time was observed at a minimum and maximum dose of ethanol extract of *Neolmarckia cadamba* treated group of animals. Ethanol extract at 100mg/kg and 200mg/kg significantly reduced the duration of immobility time in rats. After administration of a high dose of test extract (200mg/kg) shows a significant decrease in the duration of immobility time which is equivalent to imipramine (standard) treated animals. Duration of immobility time is shown in table-4.

Table 4
Effect of NcEe-1 (100mg/kg) and NcEe-2 (200mg/kg) on duration of immobility in rFST. Values expressed as mean ± SEM, n=6, p-values, p<0.001 (NcEe-1), p<0.001 (NcEe-2) when compared with controlled treated group

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Mean Duration of Immobility Time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>168.7±5.296</td>
</tr>
<tr>
<td>2</td>
<td>Imipramine (15mg/kg)</td>
<td>91±3.425***</td>
</tr>
<tr>
<td>3</td>
<td>NcEe-1 (100mg/kg)</td>
<td>137±3.347**</td>
</tr>
<tr>
<td>4</td>
<td>NcEe-2 (200mg/kg)</td>
<td>95.17±4.475***</td>
</tr>
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</table>

Effect of NcEe on the duration of immobility time in Tail Suspension Test

There is no significant decrease in duration of immobility time seen after treatment of low dose (100mg/kg) of test extract but after administration of high dose (200mg/kg) animal’s shows statistically significant reduction in duration of immobility time. Hence tail suspension test in mice at high dose (200mg/kg) of test extract shows significant results as compared to imipramine (15mg/kg) treated animals. Results of reduction of immobility parameter shown in table-5.
Table 5
Effect of NcEe-1 (100mg/kg) and NcEe-2 (200mg/kg) on duration of immobility in mTST. Values expressed as mean ± SEM, n=6, p-values, p<0.001 (NcEe-2) when compared with control treated group

<table>
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<th>Sr. No</th>
<th>Treatment</th>
<th>Mean Duration of Immobility (sec)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>152.0±5.323</td>
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<tr>
<td>2</td>
<td>Imipramine(15mg/kg)</td>
<td>79.83±3.554***</td>
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<tr>
<td>3</td>
<td>NcEe-1 (100mg/kg)</td>
<td>91.33±3.913**</td>
</tr>
<tr>
<td>4</td>
<td>NcEe-2 (200mg/kg)</td>
<td>80.50±2.778***</td>
</tr>
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</table>

Assessment of locomotor activity in the rat after acute treatment of NcEe

Locomotor activity was assessed for ten minutes test session during the experiment. Test animals receiving vehicle (saline solution), imipramine (15mg/kg) and ethanol extract (200mg/kg). As per the locomotor activity score, there is no significant effect on the animal was observed as compared to control group. Results of the locomotor activity score are shown in table-6.

Table 6
Effect of NcEe-1 (100mg/kg) and NcEe-2 (200mg/kg) on locomotor activity. Mean ± SEM, n=6, ns=Non-significant

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Locomotor activity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>371.2±7.922</td>
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<tr>
<td>2</td>
<td>Imipramine(15MG/KG)</td>
<td>359.5±4.766ns</td>
</tr>
<tr>
<td>3</td>
<td>NcEe-1 (100mg/kg)</td>
<td>347.2±3.027ns</td>
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<tr>
<td>4</td>
<td>NcEe-2 (200mg/kg)</td>
<td>358.3±8.593ns</td>
</tr>
</tbody>
</table>

Discussion

Aim of this study was to investigate a possible antidepressant activity of ethanol extract of Abrus precatorius (ApEe) and Neolmarckia cadamba (NcEe) leaves by using a behavioral animal model of depression. This study represents the antidepressant activity of the above plant extract in the forced swim test and tail suspension test. In addition, locomotor activity was checked to analyze motor dysfunction of prepared plant extract. A preliminary phytochemical test was used to identify active chemical constituents in the ethanol extract of both plants. After a phytochemical test of both plant extracts, it was observed the active constituents flavonoids. Several studies suggested the antidepressant activity of flavonoid and their derivatives in an animal model of depression. Both plant extracts are rich in flavonoid content as per the phytochemical analysis. Hence ethanol extract of Abrus precatorius (ApEe) and Neolmarckia cadamba (NcEe) leaves subjected to evaluation of antidepressant activity in an animal model of depression. Behavioral study plays an important role in screening of antidepressant activity in rodent animals like rats and mice. In the present study we employed behavioral despair animal models namely rFST and mTST. During experiment, it was observed that after acute administration of both plant
extract in rats and mice shows dose dependent reduction in immobility time as compared with vehicle-treated animal and the standard group (Imipramine treated). In forced swim test individual rat placed in a cylinder of water from which animal cannot escape during test session. Rodent animal shows escape orientation behavior. Whenever animals behavior changes eventually into a movement that the animals keep their head above water and this situation is consider as immobility parameter. This behaviour as despair in which animals lost the motivation to perform escape-oriented behavior\textsuperscript{25}. In forced swim test after acute administration of 100mg/kg and 200 mg/kg ethanol extract of \textit{Abrus precatorius} shown dose-dependent and a statistically significant reduction in duration of immobility at 200mg/kg dose, although it is statistically insignificant at 100mg/kg as compared to imipramine (shown in table-1). However after treatment of 100mg/kg 200 mg/kg of NcEe it shows a statistically significant reduction in immobility (Shown in table-4). Tail suspension test in mice is minimum stressful than forced swim test and it has greater sensitivity. In this test mouse individually suspended on a horizontal stand by their tail for a six-minute test session to observe immobility\textsuperscript{26}. During the experiment, we observed a dose-dependent reduction in immobility after administration of \textit{Abrus precatorius} leaf extract at doses 100 mg/kg and 200mg/kg.(Shown in table-2). \textit{Neolmarckia cadamba} leaf extract also shows a statistically significant reduction in immobility duration at 100mg/kg and 200mg/kg doses compared to the control treated and standard treated groups (shown in table-5). Locomotor activity was assessed by using actophotometer in the rat after acute administration of ApEe and NcEe at dose 100mg/kg and 200mg/kg test extract. (Show in table 3 and 6). Results of the present study shows the dose-dependent antidepressant-like effect after acute oral treatment of ethanol extract of \textit{Abrus precatorius} and \textit{Neolmarckia cadamba} in rFST and mTST by increasing the duration of immobility time in an animal model of depression.

**Conclusion**

Phytochemical screening indicates the presence of flavonoid in test formulation of ethanol extracts of \textit{Abrus Precatorius} (ApEe) and \textit{Neolmarckia Cadamba} (NcEe). As flavonoids play a major role in stress-induced depression. During the pharmacological evaluation, both extracts significantly decrease in immobility time of rat force swim test and mice tail suspension test at dosages 100 mg/kg and 200mg/kg of body weight, hence the ethanol extract of both plants possess dose-dependent antidepressant activity in the animal behavior model.

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**References**


