PHYTOCHEMICAL SCREENING AND NEUROPHARMACOLOGICAL EVALUATION OF ABRUS PRECATORIUS AND NEOLMARCKIA CADAMBA LEAF EXTRACT IN ANIMAL MODEL OF DEPRESSION.

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Abstract:

Abrus Precatorius and Neolmarckia Cadamba are Indian medicinal plants which referred in medicinal system of Ayurveda for treatment of various diseases. In the present study ethanol extract of Abrus Precaorius and Neolmarckia Cadamba leaves extract studied for its antidepressant activity in acute animal model of depression. Petroleum ether, chloroform, ethanol and aqueous extracts are freshly prepared from green shade dried leaves of Abrus Precatorius and Neolmarckia Cadamba. Ethanol extract of both plant subjected to phytochemical screening to detect active phytoconstituents. The phytochemical study reveals the presence of flavonoid, as flavonoid have diverse effects on improvement of mood. It plays important function in stress induced depression, hence in this study include pharmacological evaluation for antidepressant effect of ethanol extract of both plants which examined separately by using two animal behavior models which include rat Forced Swim Test (rFST) and mice Tail Suspension Test (mTST). Actophotometer use to examine effect of extract on locomotor activity rats. Comparative profile of the test formulation of flavonoid rich 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 .Ethanol extract of Abrus Precatorius shows significant reduction of immobility time in rFST(Forced Swim Test) at 200 mg/kg, however it was significant with 100 mg/kg and 200mg/kg in mTST(Tail Suspension Test).Ethanol extract of Neolmarckia Cadamba shows significant immobility in rat forced swim test at 100mg/kg and 200 mg/kg body weight. However it significantly reduced immobility in tail suspension test only at high dose 200 mg/kg body weight. Locomotor activity in rat was evaluate by using actophotometer after acute oral administration of ApEe and NcEe test extract at dosage 100mg/kg and 200mg/kg. There is no change of motor dysfunction was observed in locomotor score. Phytochemical screening indicate presence of flavonoid in test formulation of ethanol extracts of Abrus Precatorius (ApEe) and Neolmarckia Cadamba (NcEe).As flavonoid play major role in stress induced depression. During neuropharmacological evaluation both extracts shows significant reduction of immobility time in rFST(rat Forced Swim Test) and mTST(mice Tail Suspension Test) at dosages 100 mg/kg and 200mg/kg respectively, hence the ethanol extract of both plant possess antidepressant activity in animal behavior model.

Keywords: Abrus Precatiorrous, Neolmarckia Cadamba, Phytochemical screening, Flavonoid, Locomotor activity, Antidepressant effect.

1. Introduction:

The nervous system is one of the smallest and complex systems of human body which consist billion of nerve cells. Together with endocrine system, it maintains homeostasis as well as coordinates and regulates functions of human body. The nervous system is also responsible for behavior, memories, and perceptions

and initiates the voluntary movement[1].The neurotransmitters like serotonin, acetylcholine, norepinephrine, etc. plays important role to regulation of functions of nervous system. The Monoamino oxidase (MAO) is mitochondrial enzyme which found in the nerve cell. This MAO is act as safety valve because it deaminate and inactivate neurotransmitters like norepinephrine, dopamine, and serotonin etc. and cause the neurotransmitter deficiency. Neurotransmitter deficiency leads the major depression. Major depression is complex neuropsychiatric disorders which refer to pathological changes in mood state and characterized by symptoms like sad mood, psychomotor retardation, loss of interest etc[2].Today there are several synthetic antidepressant drugs are available but these drugs have restriction and limitation in clinical use due to adverse effect, hence to avoid or minimize the sever adverse effect and toxicity of synthetic drug it is necessity to find out alternative antidepressant which obtain from natural sources like herbal medicine which are used traditionally in Ayurveda. Ayurveda is traditional medicinal system of India which consist various types of medicinal herbs to prevent and treat the various disease or disorders. 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 has been assessed in variety of experimental animal models[3].Abrus precatorius and Neolamarckia cadamba are traditionally used medicinal plant as per Ayurveda. These plants were use traditionally to treat various diseases. The ethanol extract of both plants shows the presence of flavonoid after phytochemical testing. As flavonoids have central nervous system disorders which plays important role in stress induces depression. Hence the present study was undertaken to investigate the effect of flavonoid rich ethanol extract of Abrus precatorius and Neolamarckia cadamba in different animal models of experimentally induce acute depression. When results are significant and encouraging, the investigated product will be subjected to structural elucidation of flavonoid and its derivatives and investigated product will be useful in management of depression. The extracts of Abrus precatorius and Neolamarckia cadamba may have antidepressant activity, which shows their potential to be used as herbal antidepressant drug.

2. Literature Review:

2.1 Abrus precatorius[5-10]:

In Sanskrit this plant is known as Gunja (Rati). In English it called jequirity bean(rosary pea).This plant consist flowers and it belonging to family Fabaceae **.**Roots, leaves, seed of this plant was majorly use as Ayurveda medicine. White variety of this plant use to prepare oil which claim to be aphrodisiac. Tea from its leaves use for fever, cough and cold. Mixture of leaves and honey use to treat swelling. In Ayurveda these plants use to promote hair growth. Ethanol extract of Abrus precatorius was found to have analgesic, anti-inflammatory as well as antioxidant effect in animal studies.Methanolic extract of seeds shows reversible alteration in estrous cycle pattern and block ovulation in rats. Chloroform, methanol and aqueous extract of this plant shown potent antibacterial activity. An antidiabetic activity of dried leaves of Abrus precatorius developed by in in-vivo approach (Narendra Boggula, 2018). Mir Z. Gul et al. (2013) developed antioxidant and antiproleferative activity of Abrus precatorius leaf extracts –an in-vitro study.in-vitro antioxidant activity of ethanolic extract of Iraqi Abrus precatorius Linn. Stated by Zahraa Suhail Nassir, 2017). Abrus precatorius seed oil was proven for hair growth promoter in female wister albino rats (Sukirti upadhyay, 2013). Abrus precatorius seed extracts shows potent antimicrobial activity (Varaprasad Bobbarala, 2009). Ethanol leaves extract of Abrus precatorius L useful in the management of pain, psychiatric and neurological conditions: an in-vivo study (Sumanta Mondal et al. (2007).

Antiasthamatic related properties of Abrus precatorius leaves on various model developed (Dnyaneshwar J.Taur et al. (2017).Studies on antidiarrheal activity of Abrus precatorius seeds stated by O.F.C.Nwodo et al. (1991).

2.2 Neolamarckia cadamba[11-15]:

Kadamb is Indian name of this plant. In English it is commonly known as bur flower tree. It is topical tree which consist scented orange flowers in dense globe shaped cluster. This plant is belonging to family Rubiaceae. These plants find its application in Ayurveda medicinal system. Paste obtain from its leaves use to treat mouth ulcer and dyspepsia. Decoction of leaves use for gargling in stomatitis. Dried powder of leaves use as anthelmintic. Alcoholic extract of leaves possess anti-inflammatory, antimicrobial, wound healing, antibacterial, antifungal activity.Antioxidant activity of *Anthocephalous cadamba* by using solvent extracts (Alekhya V, 2013). Bark extract of Neolamarckia cadamba (Roxb.) Bosse shows Diuretic and laxative property (Mondal S, 2009). Analgesic and anti-inflammatory activities of *Anthocephalus cadamba* Roxb leave in Wister rats proven by Bachhav RS *et al. (2009).* Membrane stabilizing, anthelmintic, antioxidant activity of *Neolamarckia cadamba* fruit extract developed by Tairin Islam *et al*. (2015). Stem bark of *Neolamarckia cadamba* shows Antidiabetic activity in alloxan induced diabetic rats (Bussa SK, 2010)

3. Objectives:

To perform preliminary phytochemical test for assessment of active phytoconstituents and screening of antidepressant potential of ethanol extracts of *Abrus precatorius* and *Neolamarckia cadamba* by using acute animal models of depression.

4. Material and method:

4.1 Collection, Authentication and Processing of plant material[16-18]:

Fresh leave of *Abrus precatorious* were collected in November-2020 from geographical region of Ahmednagar Districts (Maharashtra, India). Fresh leave of *Neolmaeckia cadamba* were collected in December-2020 from geographical region of Pune Districts of Maharashtra state. .both plant was authenticating from 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.

4.2 Preparation of extract:

The powder material of *Abrus precatorious* and *Neolmaeckia cadamba* was subjected 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.

4.3 Preliminary phytochemical screening[18-19]:

Prepared ethanol extract of *Abrus precatorious* and *Neolmaeckia cadamba* was subjected for preliminary phytochemical investigation by using qualitative chemical tests. Preliminary phytochemical test of ethanol extracts of both plants shows presence of alkaloids, flavonoids, saponin glycosides, carbohydrates, steroid, terpenoids etc. (Table 1). The powder material of *Abrus precatorious* and

Neolmaeckia cadamba was subjected 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.Prepared ethanol extract of *Abrus precatorious* and Neolmaeckia *cadamba* was subjected for preliminary phytochemical investigation by using qualitative chemical tests.

4.3.1 Test for carbohydrates:

Molisch's test (General test): To 2-3 ml of extract add few drop of α-naphthol, shake it well and to it add concentrated H_2SO_4 from side of test tube. At junction of two liquid observe for violet ring.

4.3.2 Test for alkaloids:

a. **Dragendroff's test:** To 2-3 ml filtrate added few drops Dragendroff's reagent and was observed for orange brown precipitate.
b. **Mayer's test:** 2-3 ml filtrate with few drops Mayer's reagent was observed for precipitate.
c. **Hager's test:** 2-3 ml filtrate with Hagers reagent was observed for yellow precipitate.
d. **Wagner's test:** 2-3 ml filtrate with few drops of Wagner's reagent was observed for reddish brown precipitate.

*4.3.3 Test for glycosides***:**

a. **Baljet's test:** A thick section shows yellow to orange color with sodium picrate.
b. **Legal's test:** Add 1ml pyridine and 1 ml of sodium nitroprussides to aqueous or alcoholic extract, observe pink to red coloration.
c. **Raymond's test:** Test solution hot with methanol alkali solution and observe for violet coloration.

4.3.4 Test for flavonoids:

a. **Shinoda test:** Add 5 ml 95% ethanol to dry powder of extract, to it add 1-2 drop of conc. HCL and 0.5 gm. magnesium turnings. Observe orange, pink and purple color for flavonols, dihydro derivative and xanthene.
b. **Heat test solution with zinc and HCL and observe for red coloration.**
c. **To small quantity of residue add lead acetate solution till formation of yellow color precipitate.**

4.3.5 Test for fats and oils:

a. Solubility test: oils are soluble in ether, benzene and chloroform but insoluble in 90%ethanol and water.
b. Filter get paper permanently stained with oil.

4.3.6 Test for tannins and phenolic compounds:

a. Add following reagents to 2-3ml of extract:
   i. **Lead acetate solution**: Deep blue color appears.
   ii. **Bromine water:** Discoloration of bromine water.
   iii. **Acetic acid solution:** Red color appears.
   iv. **Dilute iodine solution:** Transient red color appear.

5. Pharmacological evaluation[20]:

5.1 Experimental animals:

Albino Wister rat (160-180gm), Albino Swiss mice (18-30 gm.) were used for experiment. IAEC approval obtained from IAEC of B. R. Nahata College of Pharmacy, Mandsaur, M.P as project proposal no. IAEC/BRNCOP/2020/004.

5.2 Force Swim Test in rats:

Forced swim test in rats was performed (according to Porsolt et al. 1977, 1978) at laboratory condition, one day before experiment rats are brought to laboratory. Rats use during experiment individually forced to swim inside acrylic cylinder which contains water at $25^{0\ c}$ temperature. Rat forced swim test was conduct for 15 min. test session. Change in duration of immobility time was observed and record for individual rat. Ethanol extracts which obtained from leaves of Abrus precatorious and Neolmaeckia cadamba use for acute study.to perform forced swim test rats of either sex weigh about 160-180gm were divided into four groups (n=6).First group(control/vehicle) administered saline solution(0.2ml/animal),second group administered Imipramine (15mg/kg) ,third group administered test extract of low dose (100mg/kg),fourth group administered test extract of high dose (200mg/kg).Oral route of drug administration was employed for experiment. Rat forced swim test consist two parts, part one is pretest and part two is main test.During pre-test ,rats force to swim for fifteen minutes at constant temperature in water, after fifteen minutes of test session animal were dried by using clean cloth and place into clean cage. After 24 hours of pretest main test was conducted. During main test session last six minutes are considering to observe and measure immobility parameter in rats. Immobility defines as floating in water without struggling and trying to climbing movement to escape from water.

5.3 Mice Tail Suspension Test:

This test use to evaluate antidepressant activity (described by Steru et al. 1985) .In this test animal was hanged by tail to horizontal bar and distance from floor maintains about 50cm from floor).Ethanol extract of Abrus precatorious and Neolmaeckia cadamba tested to 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 experiment. Control group receive saline solution (0.2ml/animal), second group administered imipramine (15mg/kg), and third group administered ethanol test extract at low dose (100mg/kg). Fourth group administered ethanol test extract (200mg/kg). After one hour of administration mice tail suspension test was performing as per procedure and record duration of immobility (in seconds) during last four minutes of test session. Immobility parameter was considered when mice passively hang without any movement and motions.

5.4 Assessment of locomotor activity[21]:

Drugs act on central nervous system affect the loco motor activities in human as well as animals. Locomotor activity check to determine wakefulness of mental activity; hence actophotometer test was perform to determine the effect of test extract on locomotor activity in rats. During this activity animals treated with test extract at dose 100mg/kg and 200mg/kg and compare activity with control (saline) and standard (imipramine) treated group of animal. During experiment individual animal placed into actophotometer for 10 minutes. Locomotor activity score recorded as on digital display of actophotometer.

6. Statistical analysis:

Statistical analysis was done by using ANOVA (One way Analysis of Varriance) followed by Dunnet's test. Level of significance was fixed at *P<0.05. All data express as mean ± standard error mean (SEM)

7. Results and discussion:

7.1 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, etc.is shown in table-1. (Marked as '+' and '-' signs.)

Table: 1 Preliminary phytochemical testing of ethanol extract of leaves of *Abrus precatorius and Neolmarckia cadamba.*

Sr.No	Test for Phytoconstituents	Ethanol extract of *Abrus precatorius* (ApEe)	Ethanol extract of *Neolmarckia cadamba* (NcEe)
1	Carbohydrates	+	+
2	Fats and oil	-	+
3	Alkaloids	++	+++
4	Glycosides	+	++
5	Flavonoids	+++	++
6	Tannin and phenolic compounds	+	+

7.2 Acute effect of ethanol extract of Abrus precatorius leaves on duration of immobility in rat force swim test:

After 24 hours of preliminary test immobility parameter was recorded for test extract at dose 100mg/kg and 200mg/kg. In low dose of ApEe (100mg/kg) treated animals there is no significant reduction in duration of immobility as compare to control/saline group. Imipramine (Standard) and high dose of ApEe (200mg/kg) treated animal shows more significant ($P<0.0001$) reduction in duration of immobility time when compare to control/saline treated group. Statistically significant reduction in duration of immobility time was observed and record in low and high dose of ethanol extract of Abrus precatorius treated group of animals. The result shows dose dependent effect. In experiment high dose (200mg/kg) of ethanol extract shows significant effect as compare to standard treated (imipramine (15mg/kg) animal. Imipramine shows superior effect as compare to 100mg/kg (Low dose) of ethanol extract, hence high dose (200mg/kg) of extract can be considered as antidepressant dose. Immobility time parameter is explained in table -2 and graphical presentation of experiment as shown in figure-1.

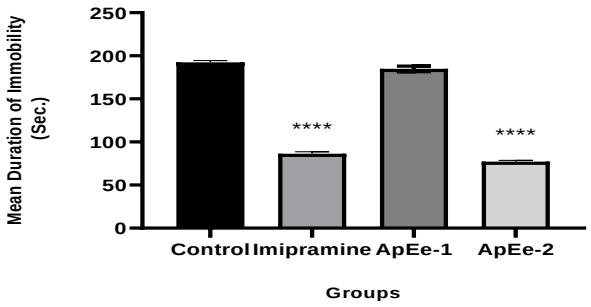
Table: 2 Comparative profile of immobility parameter (sec.) in rat forced swim test after acute treatment of ApEe-1(100mg/kg) and ApEe-2 (200mg/kg) of ethanol extract of *Abrus precatorius*.

Sr. No	Treatment	Mean Duration of Immobility Time(sec.)
1	Control	192.3±2.076
2	Imipramine(15mg/kg)	86.33±2.305***

3	ApEe-1 (100mg/kg)	132.2±8.968^{ns}
4	ApEe-2 (200mg/kg	77.17±1.537***

One way ANOVA test followed by Dunnet's test, all observed values express mean ± SEM in sec. (n= 6), P<0.0002***, P<0.0003***

Figure-1. Comparative profile of Immobility Parameter in Rat Forced Swim Test after acute treatment of 100mg/kg and 200 mg/kg of ethanol extract of *Abrus precatorius.*



7.3 Tail Suspension Test in mice:

Acute effect of ethanol extract of Abrus precatorius leaves on duration of immobility time in mice tail suspension test.

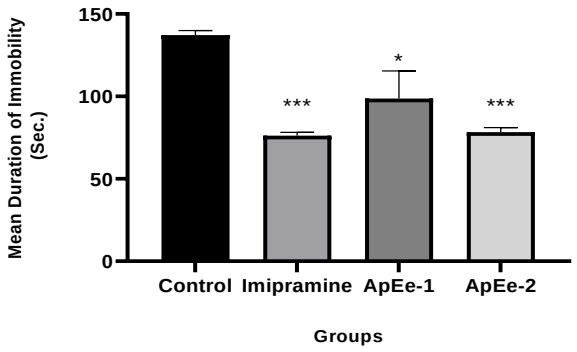
Acute effect of 100mg/kg 200mg/kg of extract is summarized in table -3.Immobility time was observed for last 4 minutes of test session. Both doses significantly reduced immobility time (P<0.0138, P<0.0003).The effect of high dose200mg/kg) was nearly equivalent as compare to imipramine (Standard treated animal).Effect of low dose (100mg/kg) also shows significant reduction in duration of immobility in mice but minimum as compare to high dose.Decrease in immobility time parameter is explained in table -3 and graphical presentation of experiment as shown in figure-2.

Table: 3 Comparative profile of immobility parameter (sec.) in mice tail suspension test after acute treatment of ApEe-1(100mg/kg) and ApEe-2 (200mg/kg) of ethanol extract of Abrus precatorius.

Sr. No	Treatment	Mean Duration of Immobility Time (sec.)
1	Control	137.2±2.750
2	Imipramine(15mg/kg)	76.17±2.056***
3	ApEe-1 (100mg/kg)	98.67±16.77*
4	ApEe-2 (200mg/kg	78.33±2.716***

One way ANOVA test followed by Dunnet's test. All values express mean ± SEM in sec. (n= 6), P<0.0138***, P<0.0003*

Figure-2. Comparative profile of Immobility Parameter in Mice Tail Suspension Test after acute treatment of 100mg/kg and 200 mg/kg of ethanol extract of *Abrus precatorius.*



7.4 Assessment of locomotor activity in rat after acute treatment of ethanol extracts of Abrus precatorius.

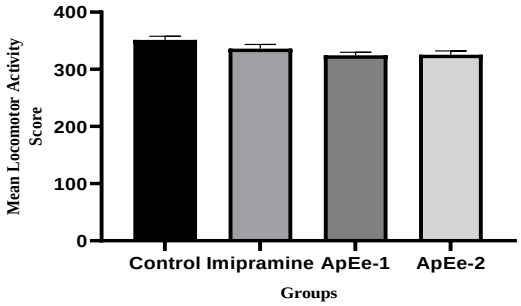
Locomotor activity score observed and recorded after single dose of administration by using actophotometer. Locomotor activity was assessed for ten minutes test session during experiment test animals receiving vehicle (saline solution), imipramine (15mg/kg), and ethanol extract (200mg/kg).As per locomotor activity score there is no any significant effect on animal was observed as compare to vehicle treated animal. Results of activity shown in table-4 and figure-3.

Table: 4 Assessment of locomotor activity of ethanol extracts of *Abrus precatorius.*

Sr. No	Treatment	Locomotor Activity Score
1	Control	351.3±6.505
2	Imipramine(15mg/kg)	336.2±7.181^{ns}
3	ApEe-1 (100mg/kg)	324.3±5.445^{ns}
4	ApEe-2 (200mg/kg	325.3±6.712^{ns}

One way ANOVA test followed by Dunnet's test. All values express mean ± SEM in sec. (n= 6), P<0.0214, P<0.0271.

Figure-3:Comparative profile of change in locomotor activity in rats after acute treatment of 100mg/kg and 200mg/kg ethanol extract of Abrus precatorius.



7.5 Acute effect of ethanol extract of Neolmarckia cadamba leaves on duration of immobility in rat force swim Test,

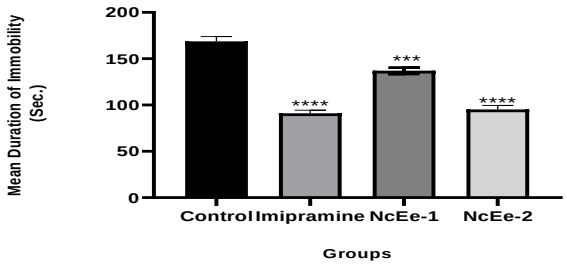
Statistically significant decrease in immobility time was observed at minimum and maximum dose of ethanol extract of Neolmarckia cadamba treated group of animals.Ethanol extract at 100mg/kg and 200mg/kg significantly reduce duration of immobility time in rats. After administration of high dose of test extract (200mg/kg) shows significant decrease in duration of immobility time which is equivalent to imipramine (standard) treated animals. Duration of immobility time is shown in table-5 and figure-4

Table: 5 Comparative profile of immobility parameter (sec.) in rat force swim test after acute treatment of NcEe-1(100mg/kg) and NcEe-2 (200mg/kg) of ethanol extract of Neolmarckia cadamba.

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

One way ANOVA test followed by Dunnet's test. All value express in mean ± SEM in sec. (n= 6), P<0.0001, P<0.0001.

Figure-4. Comparative profile of Immobility Parameter in Rat Forced Swim Test after acute treatment of 100mg/kg and 200 mg/kg of ethanol extract of *Neolmarckia cadamba*.



7.6 Acute effect of ethanol extract of *Neolmarckia cadamba* leaves on duration of immobility time in Tail Suspension Test.

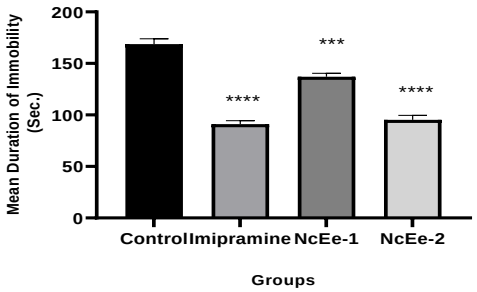
There is no significant decrease in duration of immobility time seen after treatment of low dose (100mg/kg) of test extract but after administration of high dose (200mg/kg) animal's shows statistically significant reduction in duration of immobility time. Hence tail suspension test in mice only at high dose (200mg/kg) of test extract shows significant result as compare to imipramine (15mg/kg) treated animals. Results of reduction of immobility parameter shown in table-6 and figure-5

Table: 6 Comparative profile of immobility parameter (sec.) in mice tail suspension test after acute treatment of NcEe-1(100mg/kg) and NcEe-2 (200mg/kg) of ethanol extract of *Neolmarckia cadamba*.

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

One way ANOVA test followed by Dunnet's test. All value express mean ± SEM in sec. (n= 6), P<0.0001, P<0.0001.

Figure-5. Comparative profile of Immobility Parameter in Mice Tail Suspensuin Test after acute treatment of 100mg/kg and 200 mg/kg of ethanol extract of *Neolmarckia Cadamba*.



7.7 Assessment of locomotor activity in rat after acute treatment of ethanol extract of *Neolmarckia cadamba*.

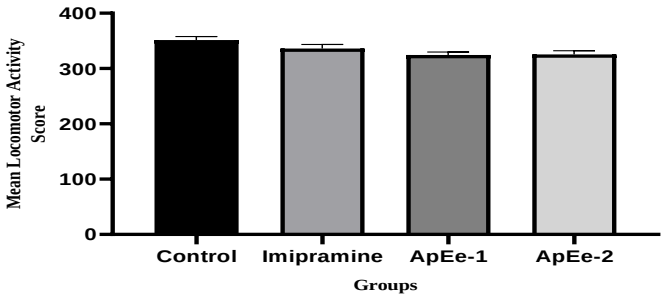
Locomotor activity score observed and recorded after single dose of administration by using actophotometer. Laocmotor activity was assessed for ten minutes test session during experiment. Test animals receiving vehicle (saline solution), imipramine (15mg/kg), and ethanol extract (200mg/kg).As per locomotor activity score there is no any significant effect on animal was observed as compare to vehicle treated animal. Results of locomotor activity score shown in table-7 and figure-6.

Table: 7 Assessment of locomotor activity of ethanol extract of *Neolmarckia cadamba*.

Sr. No	Treatment	Locomotor activity score
1	Control	371.2±7.922
2	Imipramine(15MG/KG)	359.5 ± 4.766^{ns}
3	NcEe-1 (100mg/kg)	347.2 ± 3.027^{ns}
4	NcEe-2 (200mg/kg	358.3 ± 8.593^{ns}

One-way ANOVA followed by Dunnet's test, all values mean ± SEM in sec. (n= 6), P<0.0429, P<0.3837.

Figure-6:Comparative profile of change in locomotor activity in rats after acute treatment of 100mg/kg and 200mg/kg ethanol extract of Neolmarckia cadamba.



8. Conclusion:

Ethanol extracts of *Abrus Precatorius* (ApEe) and *Neolmarckia Cadamba* (NcEe) consist flavonoid, glycoside, alkaloids, carbohydrates, tannins, fats, and oil. During experiment both plants extracts significantly decrease in immobility time in rFST and mTST at low dose (100 mg/kg) and high dose (200mg/kg) of body weight, hence the flavonoid-rich ethanol extract of both plant possess antidepressant activity in animal behavior model.

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