

NEUROPHARMACOLOGICAL EVALUATION FOR ANTIDEPRESSANT ACTIVITY OF FLAVONOID RICH ETHANOL EXTRACT OF *NEOLAMARCKIA CADAMBA* AND *ABRUS PRECATORIOUS* LEAVES IN RESERPINE INDUCED DEPRESSION IN RAT

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Abstract:

Depression is state of low mood and loss of interest in usual activities, which results in cognitive dysfunction like learning and memory impairment, affect behavior pattern of person. In the present study flavonoid rich ethanol extract of *Neolamarckia acadamba* (NcEe) and *Abrus precatorious* (ApEe) leaves was evaluated for antidepressant potential in different animal models like Forced swim test in rat (r-FST), mice tail suspension test (m-TST) and Reserpine Induced Hypothermia in Rat (r-RIH). Imipramine (15mg/kg, i.p.) was used as standard drug and reserpine (2 mg/kg, s.c.) in reserpine induced depression in rat. Duration of immobility was examined in rat forced swim test and mice tail suspension test. Rectal temperature was noted in reserpine induced hypothermia in rat. In RIH animal model rectal temperature along with ptosis was examined. In this experiment, Imipramine, NcEe and ApEe extract significantly reduced duration of immobility in both r-FST and m-TST as compare to control group. ApEe extract (Flavonoid) showed significant decrease in duration of immobility time in behavioral model of rat and mice at dose 200mg/kg and 400mg/kg, p.o. In reserpine induced hypothermia model, ApEe extract showed significant reversal of hypothermia which induced by reserpine in dose dependent manner at dose 200mg/kg and 400mg/kg, p.o. NcEe extract (Flavonoid) shows significant decrease in duration of immobility time in behavioral model and significantly showed reversal of hypothermia in reserpine induced hypothermia model at high dose (400mg/kg) as compare to vehicle and imipramine (standard) treated group.

Keywords: Antidepressant, *Neolamarckia acadamba*, *Abrus precatorious*, Reserpine-Induced Hypothermia, Forced swim test, Tail Suspension Test.

Introduction

Depression is most prevalent mental disorder which characterized by low mood and affect persons behavior, thoughts, feelings, sense of well-being and psychomotor retardation¹. Generally depression affect quality of life and it is commonly associated with dysfunctional mechanism of neurotransmitters².

Psychiatric illness is often associated with suicide which is about 10 to 20 million every year³. Depression is mainly characterized by apathy, retardation in thinking and activity, loss of energy, feeling of gloominess, suicide and despair thoughts⁴. Today there are many antidepressant drugs are available like tricyclic antidepressant, selective reversible inhibitor of monoamine oxidase (MAO), selective serotonin reuptake inhibitors (SSRI'S), Selective nor-adrenaline reuptake inhibitors (SNRI'S). But most of synthetic antidepressants have severe defects like narrow spectrum, adverse reaction, higher drug price. Basic neurological science offers promising of improvement and understanding of disease⁵.

Pathologically identifying novel mechanism which cannot be targeted by effective pharmacotherapies and screening of herbal sources of drugs. This consideration is undertaking for search of new antidepressant drugs with less side effect, wider margin of safety, fast onset of action.

In India Ayurveda is most traditional system of medicine which use for treatment of various diseases. There are various Indian medicinal plants are being used as alternative medicine for management of depression and mood disorder⁶.

On the basis of above information in present study ethanol extract of *Neolamarckia cadamba* (NcEe) and *Abrus precatorious* (ApEe) leaves was undertaken to evaluate antidepressant potential in different animal models like forced swim test in rat, tail suspension test in mice and reserpine induced hypothermia in rat^{7,8}.

Reserpine is Indole alkaloid which obtained from Rawalfia which act as sympatholytic and as sedative agent. Clinical trial shown that reserpine causes major depression after chronic administration. Reserpine mediated depression cause due to depletion of monoamines in brain^{9,10}.

In this model depletion of catecholamine like norepinephrine, epinephrine, dopamine and serotonin may lead to morphological changes in animals which induce ptosis and hypothermia, hence this model used in numerous experiment to examine and compare symptoms of depression in animals to those of human which help to determine efficacy of antidepressant medication¹¹.

On the basis of above information ethanol extract of leaves of *Neolamarckia cadamba* and *Abrus precatorious* selected for evaluation of antidepressant activity due to its traditional use in management of various diseases. Both medicinal plant consist chemical constituents like alkaloids, flavonoids, glycosides, carbohydrates, steroid, triterpenoids etc^{12,13}.

Abrus precatorious reported to possess medicinal values such as to treat leukoderma, wounds, skin diseases, asthma, cancer, sedative etc^{14,15}.

Neolamarckia cadamba use to treat wounds, pain, swelling, diabetes, mouth ulcer, urinary tract infection, renal calculi, muscular pain, and irritable bowel syndrome¹⁶.

There is no scientific report for antidepressant activity of these plant species, hence present study undertaken to evaluation of effect of *Neolamarckia cadamba* and *Abrus precatorious* leaves extract in animal models of depression.

Material and methods

Plant collection

Fresh leaves of *Neolamarckia cadamba* and *Abrus precatorious* collected from geographical region of Maharashtra, India. Both plants were authenticated from Biological Survey of India, Pune. Leaves of both plants were clean by pure water and shade dried for two week at room temperature¹⁷.

Preparation of extract and phytochemical screening.

Preliminary phytochemical screening

Preliminary phytochemical screening were carried out with ethanol extract of *Neolamarckia cadamba* and *Abrus precatorious* leaves for qualitative identification of active phytoconstituents using standard procedure^{19,20}.

HPTLC analysis for flavonoid:

HPTLC method use for fingerprinting, identification of compound and to determination of class of compound from ethanol extract of *Neolamarckia cadamba* and *Abrus precatorious*.

HPTLC method:

Sample preparation: - 10mg/ml in methanol.

Stationary phase: TLC silica gel 60 F254 by Merck (Cat. No. 1.05554.0007)

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Mobile phase:

- i. **For Steroids:** n-Butanol: Methanol: Water (3:1:1v/v/v)
- ii. **For Glycosides:** Ethyl acetate: Methanol: Water (81:11:8)
- iii. **For Essential oil:** Toluene: Ethyl acetate (9.3:0.7)
- iv. **For Saponins:** Chloroform: Acetic acid: Methanol: Water (6.4:3.2:1.2:0.8)
- v. **For Triterpenoids:** n-Hexane: Ethyl acetate: (1:1)
- vi. **For coumarins:** Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26)
- vii. **For Bitter Principle:** Ethyl acetate: Methanol: Water (7.7:1.5:0.8)
- viii. **For Tannins:** Toluene: Ethyl acetate: Formic acid (6:4:0.3)
- ix. **For Flavonoids:** Ethyl acetate: Water: Formic acid: Acetic acid (100:26:11:11)
- x. **For Alkaloids:** Toluene: Ethyl acetate: Methanol: Ammonia 25% (30:30:15:1)

Developing distance: 70mm from the lower edge of the plate.

Derivatizing reagent:

- i. **For Flavonoids:** 10% methanolic Sulphuric acid.
- ii. **For Steroids, Glycosides, Essential oil, Saponins, Triterpenoids, coumarins, Bitter Principle:** Anisaldehyde Sulphuric Acid Reagent.
- iii. **For Alkaloids:** Dragendorff's Reagent.
- iv. **For Tannins:** Iron chloride (FeCl₃).
- v.

Separation of flavonoid

Shade dried leaves of both plants was grind and convert into coarse powder. Powder material (500 gm.) was defatted by maceration with hexane for 24 hours. After filtration defatted plant material allow to dry at room temperature. The defatted plant material was extracted with 80% ethanol by soxhlet apparatus until complete exhaustion. The ethanol extract was evaporated under reduced pressure at room temperature not exceed 40⁰c to give crude fraction. Crude extract was acidified with hydrochloric acid (5%) to pH-2 and partitioned three times with ethyl acetate to get two layers (aqueous acidic and ethyl acetate layer). Ethyl acetate layer was separated and evaporated to dryness under reduced pressure and basify with 300ml of sodium hydroxide 5% to pH-10 and extracted with chloroform in separator funnel to get two layers (aqueous basic layer and chloroform layer). Aqueous basic layer was separated and evaporated to dryness and acidified with 5% hydrochloric acid to pH-2 then extracted with ethyl acetate to get fraction designated as flavonoid. Prepared flavonoid fraction was store in well closed container for further use¹⁸.

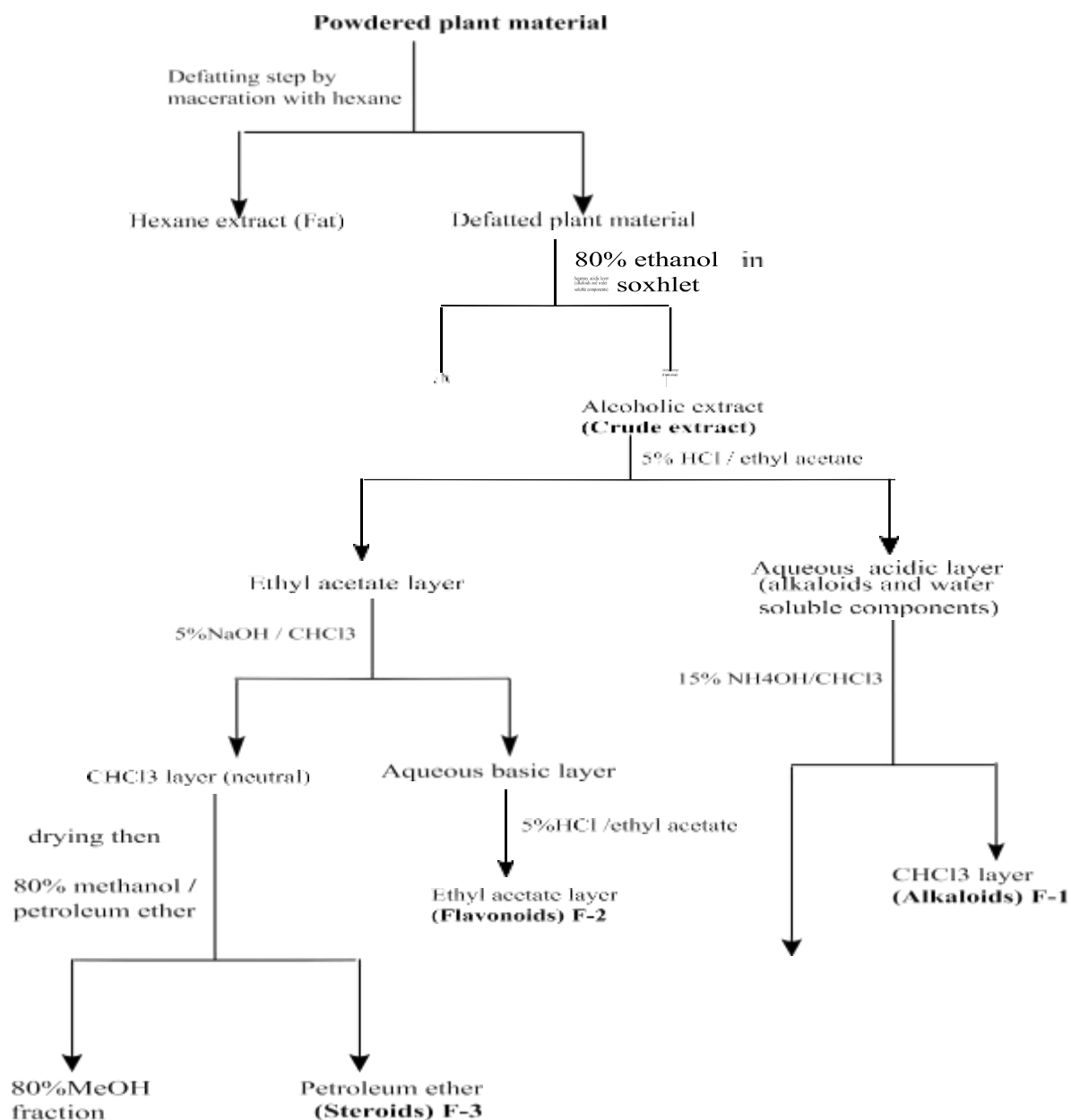


Figure 1. General scheme for separation of different plant constituents¹⁸

Pharmacological evaluation

Animals

Healthy Albino Wister rat (160-180gm) and Albino Swiss mice (18-30gm) were used in this study. Under controlled laboratory conditions, animals were exposed to a 12-hour light cycle. IAEC approval was obtained from IAEC of B.R. Nahata College of Pharmacy, Mandsaur, Madhya Pradesh as project proposal No. IAEC/BRNCOP/2020/004.

Acute toxicity study

Toxicity study of ethanol extract of *Neolamarckia cadamba* and *Abrus precatorious* were carried out in healthy Swiss albino mice which are weighing between 25-30gm. Toxicity studies perform according to OECD guideline 420. Fixed dose method was used for dose determination²¹.

Forced swim test in rat

Forced swim test was performing at laboratory condition, one day before the experiment experimental animal brought laboratory. The experiment was carried out in narrow glass cylinder which contains water at 25⁰ temperature from which they cannot escape. This test was conducted for 15 Minuit test session. All animals were fasted for 3Hrs prior administration of vehicle, standard, and test compounds (extract). 30 Minuit latter, individual animal were subjected to swim. During first two minutes animal was allowed to adjust in water condition. During test session change in duration of immobility parameter was observed and recorded for individual animal. Duration of immobility was measured with stopwatch. Immobility time was the time during which the animal floated on the surface with front paws together and made only those movements which necessary to float^{22, 23}.

Mice tail suspension test

This test is also known as dry test. In these test mice were suspended on horizontal stand and maintain 50 cm distance from the floor. Vehicle, standard drug and test compound (extract) were administered to mice 60 Minuit prior to testing. The duration of immobility was recorded for 6 Minuit's by using stopwatch. The immobility parameter was considered when mice passively hang without any movement and motions^{24, 25}.

Reserpine induced hypothermia

All animals were fasted for 3Hrs prior to administration of vehicle, standard drug, test compound (extract). The basal rectal temperature was measured by inserting a clinical thermometer to constant depth 3cm. On day before testing, animals were injected with 2mg/kg reserpine subcutaneously. Animals kept in laboratory condition and free access to food and water for 18Hrs. After 18Hrs of administration of reserpine, Rectal temperature was measured every 30 min. 1Hrs, 2Hrs, 4Hrs after oral administration of vehicle, standard drug, test compound (extract). Scoring of ptosis in mice recorded as Normal-0, 1/4th closed eyes-1, 1/2 closed eyes-2, 3/4 closed eyes-3, fully closed eyes-4²⁶.

Statistical Analysis

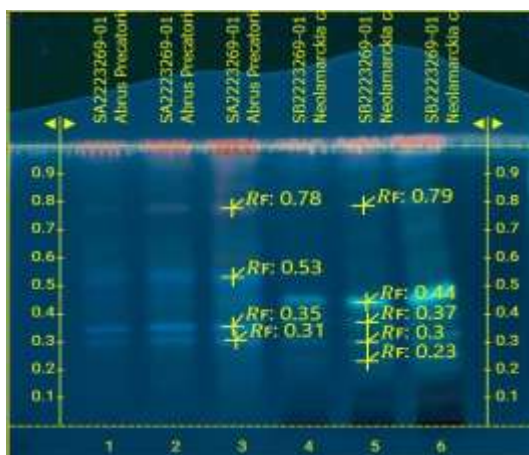
Results were presented as mean \pm SEM. The data was subjected for statistical analysis by one way analysis of variance (ANOVA) followed by Dennett's t test and $p < 0.05^*$, $**p < 0.002$ and $***p < 0.0001$ was considered as significant and $p > 0.05$ was considered as non-significant (ns) when compare with control group.

Results:**Table 1:** Phytochemical constituents of ethanol extract of *Neolamarckia cadamba* and *Abrus precatorious*.

Sr.No	Phytochemical constituents	Ethanol extract of <i>Neolamarckia cadamba</i> (NcEe)	Ethanol extract of <i>Abrus precatorious</i> (ApEe)
1	Flavonoids	++	++
2	Alkaloids	++	+
3	Steroids	+	-
4	Carbohydrates	+	+
5	Saponins	+	-
6	Triterpenoids	-	-
7	Gum and mucilage	-	+

HPTLC analysis for flavonoid:

- **Derivatized image at R366 with Rf tip:** Flavonoids are detected after derivatisation with Iron chloride in R366. Detected in R366 at Rf 0.31, 0.35, 0.53 and 0.78 in *Abrus Precatorius* (Florescent compound bands) and Rf 0.23, 0.3, 0.37, 0.44 and 0.79 in *Neolamarckia cadamba* (Florescent compound bands).

**Figure 2.** Derivatized image at R366 with Rf tip**Acute oral toxicity study:**

Fourteen animal were treated with 2000mg/kg of both plant extract by oral route. Animals were observe continuously for first 4 Hrs. for behavioral change and mortality if any at the end of 72 Hrs. Dose was found to be safe since no animal died and animal did not show any behavioral change.

Table 2: Effect of ApEe on duration of immobility time in rat forced swim test.

Sr.No	Group	Mean duration of immobility time (sec.)
1	Control	154.8 ± 4.736
2	Imipramine (15mg/kg)	98.17 ± 2.455***
3	ApEe-1 (200mg/kg)	131±5.530**
4	ApEe-2 (400mg/kg)	121.2±5.624***

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 3: Effect of ApEe on duration of immobility time in mice tail suspension test.

Sr.No	Group	Mean duration of immobility time (sec.)
1	Control	113.8 ± 4.490
2	Imipramine (15mg/kg)	77.50±2.693***
3	ApEe-1 (200mg/kg)	98.33±2.275*
4	ApEe-2 (400mg/kg)	88.33±6.286**

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 4: Effect of ApEe on temperature in reserpine induced hypothermia in rat.

Sr.No	Group	Initial temperature	Rectal temperature (mean ± SEM);N=6			
		0 Min.	30Min.	60Min.	120Min.	240Min.
1	Control	34.10±0.39	33.43±0.48	34.65±0.61	33.73±0.52	36.33±0.67
2	Imipramine(15mg/kg)	32.85±0.34	35.10±0.29*	37.98±0.76***	39.23±0.60***	39.88±0.44***
3	ApEe (200 mg/kg)	33.27±0.31	33.85±0.37 ^{ns}	35.75±0.91*	36.85±0.52**	39.50±0.58**
4	ApEe (400mg/kg)	34.20±0.58	35.50±0.50 ^{ns}	38.03±0.56**	39.90±0.46***	40.82±0.50**

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 5: Effect of ApEe on ptosis in reserpine induced hypothermia in rat.

Sr.No	Group	Initial temperature	Ptosis score; N=6			
		0 Min.	30Min	60Min.	120Min.	240Min.
1	Control	4±0	4±0	3.66±0.21	3.83±0.16	3.66±0.21
2	Imipramine(15mg/kg)	4±0	3.16±0.16***	3±0***	3±0***	2.33±0.21***
3	ApEe (200 mg/kg)	4±0	3.83±0.16 ^{ns}	3.33±0.21*	3.16±0.16**	3.33±0.21*
4	ApEe (400mg/kg)	4±0	3.66±0.21 ^{ns}	2.66±0.33**	2.33±0.21***	2.50±0.4**

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 6:Effect of NcEe on duration of immobility time in rat forced swim test.

Sr. No	Group	Mean duration of immobility time (sec.)
1	Control	218±3.183 ^{ns}
2	Imipramine (15mg/kg)	171.3±3.084***
3	NcEe-1 (200mg/kg)	216±1.248
4	NcEe-2 (400mg/kg)	196±3.008***

Results were analyzed by one way ANOVA using Dennett's multiple comparison test; significance at *** $p < 0.001$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 7:Effect of NcEe on duration of immobility time in mice tail suspension test.

Sr.No	Group	Mean duration of immobility time (sec.)
1	Control	135±2.372 ^{ns}
2	Imipramine (15mg/kg)	68.50±1.746***
3	NcEe-1 (200mg/kg)	120.3±3.593**
4	NcEe-2 (400mg/kg)	114.8±4.246***

Results were analyzed by one way ANOVA using Dennett's multiple comparison test; significance at *** $p < 0.001$, ** $p < 0.01$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 8:Effect of NcEe on temperature in reserpine induced hypothermia in rat.

Sr.No	Group	Initial temperature	Rectal temperature (mean ± SEM);N=6			
		0 Min.	30Min	60Min.	120Min.	240Min.
1	Control	33.98±0.50	34.30±0.53	32.53±0.45	34.15±0.48	34.07±0.52
2	Imipramine(15mg/kg)	33.57±0.49	35.22±0.38 ^{ns}	31.43±0.65***	37.98±0.70***	37.43±0.61**
3	NcEe (200 mg/kg)	34.81±0.43	36.60±0.18 ^{ns}	35.07±0.38 ^{ns}	36.22±0.48 ^{ns}	37.30±0.61**
4	NcEe (400mg/kg)	34.48±0.27	35.30±0.43 ^{ns}	35.20±0.33 ^{ns}	36.68±0.31***	36.53±0.39**

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Table 9: Effect of NcEe on ptosis in reserpine induced hypothermia in rat.

Sr.No	Group	Initial temperature	Ptosis score; N=6			
		0 Min.	30Min	60Min.	120Min.	240Min.
1	Control	4±0.00	4±0.00	3.66±0.21	3.66±0.21	3.50±0.22
2	Imipramine(15mg/kg)	3.83±0.16	3.00±0.25 ^{ns}	2.33±0.21***	2.66±0.33**	2.33±0.25***
3	NcEe (200 mg/kg)	4±0.00	3.83±0.16 ^{ns}	3.66±0.21 ^{ns}	3.33±0.21*	3.33±0.21*
4	NcEe (400mg/kg)	3.83±0.16	3.66±0.21 ^{ns}	2.833±0.30*	2.83±0.30*	2.66±0.21**

Results were analyzed by one way ANOVA using Dennett's multiple comparison test;significance at *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, non-significant (ns) at $p > 0.05$ when compared with control treated group.

Discussion

There are several causes of depression and many hypotheses have been proposed regarding the basis of depression like disturbances in gamma amino butyric acid transmission, monoamine and glutamate, hypothalamic-pituitary-adrenal axis hyperactivity, neurotropic factor dysfunction. Monoamines are transported into presynaptic vesicles through the vascular monoamine transporter which is blocked by reserpine. Rat with reserpine induced depression due to monoamine depletion exhibits anxiety and depression like behavior, such as increased immobility time and decreased locomotor activity in behavior tests which are relative to results observed in control group²⁷.

Flavonoid is active and potential phytoconstituent which is obtained from medicinal plants. Flavonoid possesses potential effect in treatment of neurodegenerative disorders²⁸. In the present study, flavonoid rich ethanol extract of leaves of *Neolamarckia cadamba* and *Abrus precatorious* was subjected to investigation for evaluation of antidepressant activity in animal behavior model (rat and mice) and reserpine induced depression in rat. Results of this study revealed that *Neolamarckia cadamba* and *Abrus precatorious* ameliorate depression like behavior.

In this study reserpine was used to induce reactive type of depression in rats. 2 mg/kg dose of reserpine administered to experimental animals by intraperitoneal route (i.p.) on the day before testing to induce significant hypothermia. Reserpine is a sympatholytic drug which depletes catecholamine in nervous tissue and in the brain, can irreversibly inhibit the vascular uptake of monoamines including noradrenaline, dopamine, 5-Hydroxytryptamine, which depletes monoamine in brain and produces depression like syndrome in animals. In the present study we tried to demonstrate that *Neolamarckia cadamba* and *Abrus precatorious* leaves extract are able to reverse the depression in rat which is induced by reserpine.

Flavonoid is isolated from *Neolamarckia cadamba* and *Abrus precatorious* leaves were subjected for evaluation of antidepressant activity. Primarily both plant extracts were subjected for phytochemical investigation and acute oral toxicity study. Phytochemical investigation of both plants shows presence of important constituents like flavonoid, alkaloid, carbohydrate, steroids etc.

In acute oral toxicity study, NcEe and ApEe did not show any lethal effect up to a dose of 2000 mg/kg.

Effect of NcEe and ApEe was investigated for its antidepressant activity by using various experimental animal models like mice tail suspension test (mTST), rat forced swim test (rFST) and reserpine induced hypothermia (RIH). Forced swim test and tail suspension test are most commonly used preliminary screening tests for characterizing potential antidepressant drugs.

In rat forced swim test and mice tail suspension test ApEe extract at a dose of 200 mg/kg and 400 mg/kg, per oral showed significant increase in motor activity in experimental animals which elevates depressed mood by decreasing immobility time compared to standard treated group. In reserpine induced hypothermia model, after treatment of ApEe extract at a dose of 200 mg/kg and 400 mg/kg per oral it shows significant dose dependent reversal of

hypothermia which induced by reserpine. It showed that ApEe extract may inhibit reuptake of neurotransmitters like serotonin and norepinephrine. Ptosis score was also observed during this experiment which showed significant effect as compare to control and standard (Imipramine) treated group.

After administration of NcEe at dose of 200mg/kg and 400mg/kg per oral (p.o) it shows significant decrease in duration of immobility time in rat forced swim test and tail suspension test in mice at dose of 400mg/kg as compare to standard treated group. In reserpine induced hypothermia model NcEe extract increase temperature at high dose (400mg/kg) but less significant as compare to imipramine (Standard) treated animals.

In reserpine induced hypothermia model, after treatment of ApEe and NcEe at dose of 200mg/kg and 400mg/kg shows significant antidepressant activity in experimental animal model.

Conclusion

In present experiment flavonoid extract of *Neolamarckia cadamba* and *Abrus precatorious* leaves was investigated for antidepressant activity by using various experimental animal models. ApEe extract showed significant decrease in duration of immobility time in behavioral model of rat and mice at dose 200mg/kg and 400mg/kg ,p.o. In reserpine induced hypothermia model, ApEe extract showed significant reversal of hypothermia which induced by reserpine in dose dependent manner at dose 200mg/kg and 400mg/kg ,p.o.

NcEe extract shows significant decrease in duration of immobility time in behavioral model and significantly showed reversal of hypothermia in reserpine induced hypothermia model at high dose (400mg/kg) as compare to imipramine (standard) treated group.

From all above study ,the present investigation suggest that flavonoid rich extract of *Abrus precatorious* leave possess significant antidepressant activity as compare to *Neolamarckia cadamba* extract by inhibiting reuptake of neurotransmitters like serotonin and norepinephrine.

Further investigation is necessary to identify active constituent and mechanism of action which is responsible for antidepressant activity.

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