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Evaluation of anxiolytic and anti-depressant activity of *Neolamarckia cadamba* in mice

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Abstract

Objective: Evaluation of anxiolytic and anti-depressant activity of *Neolamarckia cadamba* in mice.**Material & Method:** The aqueous and methanolic extract of "*Neolamarckia cadamba*" and chose low, medium, and high doses for therapy. The behavioural consequences of an oral acute or subacute (10 day) treatment. *Neolamarckia cadamba* (250 and 500 mg/kg, p.o) aqueous and methanolic stem bark extract assessed in male and female Swiss mice (EPM). Diazepam (1 mg/kg) will also be evaluated. Anti-anxiety drug testing in the lab.**Results:** *Neolamarckia cadamba*, acute oral toxicity was detected with different extracts (ENC & AQNC) having dose (5, 50, 300, 1000 mg/kg) via oral route, shows no change in behavioural responses and observation shows no acute oral toxicity. Hence depending upon it, Dose was selected 250 mg/kg & 500 mg/kg for our experimental work.**Conclusion:** *Neolamarckia cadamba* has both anxiolytic and antidepressant properties, which likely operate through BZD receptors, selective serotonin reuptake inhibitors. The antidepressant and anxiolytic properties of *Neolamarckia cadamba* ethanolic and aqueous extracts were investigated in swiss albino mice at doses of 250 and 500 mg/kg, respectively. Both extracts (ANC & ENC) showed strong antidepressant and anxiolytic efficacy using TST and EPM parameters.**Keywords:** *Neolamarckia cadamba*, ethanolic; methanolic, Anxiolytic and Anti-depressant activity, mice.

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Introduction

Mental disorders are predicted to account for 12% of the global burden on the human health systems. According to the Psychiatric Morbidity Survey, one in six of us would be diagnosed as having chronic anxiety or depression disorder in life time. Anxiety is one of the most common mental disorders in humans, which affects 28.8% of world population [1]. It can be considered as "intact" condition, which may interfere with even normal routine life of the person. We all experience anxiety for example, speaking in front of a

Group can make us anxious, but that anxiety also motivates us to prepare and practice. Driving in heavy traffic is another common source of anxiety, but it helps keep us alert and cautious to avoid accidents. However, when feelings of intense fear and distress become overwhelming and prevent us from doing everyday activities, this may be the cause of an anxiety disorder [2]. Anxiety disorders form one of the most common psychiatric disorders affecting both children and adults. Anxiety disorder may develop from a complex set of risk factors including genetics, brain chemistry, personality and life events.

Anxiety may be accompanied by a variable constellation of autonomic signs and symptoms such as light-headedness, perspiration, palpitations, and tightness in the chest, dyspnoea, and hypertension, tingling in the extremities, tremor, and restlessness. Depression is a common mental disorder that presents with depressed

mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration [3, 4]. It is characterized primarily by mood fluctuations, rather than by agitations of thought. It is the most common form of affective disorder, may range from a very mild condition, bordering on normality to severe depression. It can be a complex interaction of social, psychological and biological factors. In other words, depression is a major cause of disability and premature death. Major depression is a common disorder that continues to result in considerable morbidity and mortality despite major advances in treatment. We can say that mental depression can affects the person mood, physical health, behaviour and thoughts [4].

Materials and Methods

Plant materials

Collection and authentication of plant materials

The *Neolamarckia cadamba* stem bark collected from Herbal Garden of St. Soldier Institute of Pharmacy, campus and authenticated from Raw Material Herbarium and Museum, Delhi (RHMD). *Neolamarckia cadamba* stem bark shaded dried; powdered, Aqueous Extract and Ethanolic Extract prepared by soxhlet apparatus. Aqueous and Ethanolic extract of *Neolamarckia cadamba* administered in different doses (250 and 500 mg/kg, p.o) to mice.

Methods of collection of data

The data based on laboratory animal experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was carried out as per the guidelines of Committee for the purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of environment and Forests, Government of India (Reg. No. 2011/PO/Re/S/18/CPCSEA and date of registration is 1/5/2018) for the use and care of experimental animals. Adequate measures were taken to minimize pain or discomfort with animal's experimental procedure. Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2019/PR-001).

Drugs and Chemicals

All chemicals of analytical grade procured from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

Preparation of extract

Hot Continuous Extraction (Soxhlet) [5]

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in Soxhlet apparatus as shown sample

in Figure. The extracting solvent in distillation flask is heated, and its vapours condense in condenser. The condensed extract ant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid raises the top of siphon tube, the liquid contents move siphon into distillation flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

Figure 01: Hot Continuous Extraction (Soxhlet)

Ethanolic extract (ENC)

Stem bark of *Neolamarckia cadamba* shade-dried and powdered. The powder (100 g.) packed in a soxhlet apparatus and subject to continuous hot percolation for about 8 h. with ethanol (350 ml.) as solvent. The extract concentrated to a semi-solid mass under vacuum and completely be dried in desiccators.

Aqueous extract (AQNC)

Approximately 100 g. of shade-dried powder of Stem bark of *Neolamarckia cadamba* taken in a 1 L beaker and chloroform: water (1:99) added up to a sufficient level to immerse the drug completely. Chloroform added as a preservative to prevent microbial growth. This set up will place aside for 72 h. with stirring at alternate

intervals. Finally, the contents of the beaker will vacuum filtered to get a clear watery brown colored extract. The extract concentrated under high vacuum and completely dried in desiccators.

Experimental animals

Healthy, adult swiss albino mice of either sex weighing (25-30 g), maintained under standard laboratory conditions, at temperature $25 \pm 2^\circ\text{C}$ and a 12 hr light-12 hr dark period employed for the experimentation. Food and water provided ad libitum.

Acute oral toxicity study [6]

Acute toxicity study for the aqueous and methanolic extract of "*Neolamarckia cadamba*" done according to the OECD guidelines No: 423 and low, medium and high dose selected for treatment.

Method

The overnight fasted mice divided into 04 groups, each group consisting of 3 animals. The ENC & AQNC various doses (5, 50, 300 and 2000) by oral route with a gavage. After administration of the extract, the animal observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and also monitored up to 14 days for the toxic symptoms and mortality. The same repeated for AQNC in another 04 groups. After 14 days of acute oral toxicity the survival mice rehabilitated and reused for experimentation.

Experimental parameters

Anti-anxiety animal model

The behavioral effects of an acute or sub-acute (10-day course) orally administered. "*Neolamarckia cadamba*" (250 and 500 mg/kg, p.o) aqueous and methanolic extract of stem bark evaluated in male and female swiss mice by elevated plus-maze (EPM). The effects of diazepam (DZP; 1 mg/kg) will also be assessed. Laboratory models for testing anti-anxiety activity.

Elevated plus maze (EPM) [7]

The Elevated Plus-Maze test widely employed for measuring anxiolytic and anxiogenic-like activities in mice. The Elevated plus Maze for mice consisted of two open arms (16 cm $\times$ 5 cm) and two covered arms (16 cm $\times$ 5 cm $\times$ 15cm) extended from a central platform (5 cm $\times$ 5 cm) and the maze elevated to a height of 25 cm from the floor. Plants administered 30 min before start of experiment. Each mouse placed individually at the central platform of maze with its head facing towards an open arm and observed for 5 min to record the number of entries into open arm, closed arm and time spent in each arm. Entry into an arm considered valid only when

all four paws of the mouse inside that arm. The plus maze carefully wiped with hydrogen peroxide and dried with sponge after each trial. Test conducted in quite room to avoid disturbances to animals.

Figure 02: Elevated plus maze for mice

(A) Open arms; (B) closed arms

Experimental Design

Group 1: control (solvent)

Group 2: mice treated with ENC (Low dose)

Group 3: mice treated with ENC (High dose)

Group 4: mice treated with AQNC (Low dose)

Group 5: mice treated with AQNC (High dose)

Group 6: mice treated with diazepam 1 mg/kg

Antidepressant Animal model

The behavioural effects of an acute or sub-acute (10-day course) orally administered. *Neolamarckia cadamba* (250 and 500 mg/kg, p.o) aqueous and methanolic extract of stem bark evaluated in male and female swiss mice in tail suspension test (TST). The effects of fluoxetine (FXT; 10 mg/kg) will also be assessed. Laboratory models for testing anti-depressant activity.

Tail suspension test (TST)

Tail suspension test is a commonly employed model to evaluate new anti-depressant medicines⁸. Immobility reflects a state of helplessness, which can be reversed by drugs such as imipramine and fluoxetine, effective clinically in human depression. The index of depression in this experimental model is taken as the immobility duration over a specific period of time. The increase in immobility period indicates the depressed state of mind. Whereas, reduction in immobility period exhibits a depression-free state of mind. Whereas, reduction in immobility period exhibits a depression-free state of mind. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed

approximately one cm from the tip of the tail. Immobility time was recorded during a 6 min period [9]. Animal was considered to be immobile, when it did not show any movement of body and hanged passively.

Fig 03: Tail suspension test (TST)

(1) Immobility – the mouse hangs without engaging in any activity; (2) swinging – keeping its body straight, the mouse continuously moves its paws in a vertical position and/or moves its body from side to side; (3) curling – the mouse engages in active twisting movements.

Experimental Design

Group 1: control (solvent)

Group 2: mice treated with ENC (Low dose)

Group 3: mice treated with ENC (High dose)

Group 4: mice treated with AQNC (Low dose)

Group 5: mice treated with AQNC (High dose)

Group 6: mice treated with fluoxetine. 10 mg/kg

Body weight analysis

During the same time of study, weekly body weight analysis recorded.

Statistical analysis

All the results expressed as standard error of mean (S.E.M.). Data analysed using one-way ANOVA (Graph pad prism version 5.00 software) followed by suitable post-test like Dunnett's t-test. $p < 0.05$ considered as statistically significant.

Total No. of animals required:

No of the animal in each group = 06 or 07

No. of groups = 12

Total no. of animals required = $06/07 \times 12 = 80$

First of all we will complete one parameter i.e. Anxiolytic study, then after rehabilitation / washing period. We will use same animals for second parameter i.e. Antidepressant by this way. We will minimize the number of experimental animals up to 50% i.e. 40.

Results & Discussion

Acute oral toxicity study

The median lethal dose (LD_{50}) of AQNC & ENC was determined in accordance with the Organization for Economic Co-operation and Development (OECD, 425) guidelines using five mice which were fasted overnight before dosing with different extracts of AQNC & ENC separately at maximum dose level up to 1000 mg/kg orally starting from dose of 5, 50, 300 mg/kg. One mouse was initially dosed and food was further withheld for 4 h. It was observed for the first 24 h and then for 14 d for signs of toxicity (changes in mucous membranes, skin, fur and eyes, circulatory, respiratory, somato-motor activity and behaviour pattern) and mortality. It has been observed that no change in behavioural responses and observation shows any acute oral toxicity. The remaining four mice were also dosed and observed for 2 weeks. Thereafter, the LD_{50} was estimated.

Evaluation of anxiolytic and antidepressant effect of *Neolamarckia cadamba* stem bark extracts

Table 01: Anxiolytic Animal Model: Elevated plus maze (EPM) Observations and calculations

S.NO.	GROUP & DOSE	Elevated plus maze	
		Time spent in open arm(sec.)	No. of crossings
1	Control 1% w/v sodium cmc (5mg/ kg, p.o.)	60.2 ± 2.854	4.6 ± 0.376
2	Aqueous extract (Stem bark NC)		
	- 250mg/kg	210.2 ± 2.374*	12.2 ± 0.384*
	- 500mg/kg	262.4 ± 1.664*	13.6 ± 0.512*
3	Ethanol extract (Stem bark NC)		
	- 250mg/kg	198.0 ± 2.776*	12.0 ± 0.448*
	- 500mg/kg	270.8 ± 2.598*	12.6 ± 0.506*
4	Standard dose (Diazepam- 1 mg/kg)	290 ± 1.818**	16.6 ± 0.8126**

* $P < 0.05$, * $P < 0.01$, ** $P < 0.001$

When compared with the control group. All values represent = Mean ± SEM, $n = 5/6$ in each group

Figure 4: Elevated plus maze

Table 2: Anti-depressant Animal Model: Tail Suspension Test (TST) Observations and calculations

S.N.	Group & Dose	Immobility (sec.)
1.	Control 1% w/v sodium cmc (5mg/ kg, p.o.)	255 ± 4.806
2.	Aqueous extract (Stem bark NC)	
	- 250mg/kg	90.2 ± 2.384*
	- 500mg/kg	42.4 ± 1.855*
3.	Ethanollic extract (Stem bark NC)	
	- 250mg/kg	79.0 ± 2.764*
	- 500mg/kg	38.8 ± 2.696*
4.	Standard dose (Fluoxetine- 10 mg/kg)	16.2 ± 1.626**

*P < 0.05, # P < 0.01, ** P < 0.001

When compared with the control group. All values represent = Mean ± SEM, n= 5/6 in each group

Figure 5: Tail suspension method

Aqueous Extract of *Neolamarckia cadamba* stem bark (AQNC)

Ethanollic Extract of *Neolamarckia cadamba* stem bark (AQNC)

Biochemical estimation

Collection of Blood Samples

On 15th day, blood (0.3 ml) was withdrawn from tail vein from all groups of mice. Blood samples were centrifuged at 2500 rpm for 10 min using refrigerated centrifuge (Paramount scientific works, Ambala cantt, India) to separate the plasma, which was used for estimation of corticosterone levels.

Estimation of plasma corticosterone levels

The quantitative estimation of corticosterone levels in the blood plasma was performed by the method of Bartos and Pesez, 1979. To 1.0 ml of sample in ethanol, 0.50 ml of 0.10 % solution of p-nitroso-N, N-dimethylaniline in ethanol was added and the tubes were immersed in ice water for 5 min, and then 0.50 ml of 0.10 N-sodium hydroxide was added. The tubes were plugged with cotton-wool, and were let to stand at 0°C for 5 h, protected against light. To the above solution, 2.0 ml of buffer for pH 9.8, 5.0 ml of 0.10 % solution of phenol in ethanol and 0.50 ml of 1.0 % aqueous solution of potassium ferricyanide were added. The tubes were kept in a water bath at 20±2°C for 10 min. The solution was read at 650 nm using UV-visible spectrophotometer (UV 3200 UV-VIS Spectrophotometer, Somajiguda, Hyderabad).

Table 03: Groups 1 to 6 were tail bled on day 1 and then corticosterone levels were combined to obtain the average levels in tail blood. For treatment of Groups see their respective experimental design.

Groups	Group treated with Dose(mg/kg)	Mean corticosterone(ng/ml)±SE
1	Control	198.87± 0.58
2	Test Aqueous lower dose	176.48± 1.46
3	Test Aqueous higher dose	170.98±2.26*
4	Test Ethanollic lower dose	168.16±1.27*
5	Test Ethanollic higher dose	158.48±0.30#
6	Standard dose	140.82±0.36**

*P < 0.05, # P < 0.01, ** P < 0.001

When compared with the control group. All values represent = Mean $\pm$ SEM, n= 5/6 in each group

Table showing the mean ($\pm$ SE) values of corticosterone levels in Post Tail Suspension Test Experiments

It is known that stress enhances the activity of the hypothalamus-pituitary-adrenal (HPA) axis and results in increased secretion of corticosteroids from the adrenal cortex. Cortisol and corticosterone are thus often used as biomarkers for stress and depressive disorders. Although corticosterone is considered the main glucocorticoid involved in regulation of stress responses in rodents, researchers often choose to detect cortisol for stress indicators in consideration of convenience and kits availability.

Note: TALD= Test Aqueous lower dose; TAHD= Test Aqueous higher dose; TELD= Test Ethanol lower dose; TEHD= Test Ethanol higher dose; STD= standard.

Figure 6: Graph showing the corticosterone levels in Post Tail Suspension Test Experiments

Discussion

Progress in unravelling the neuro-chemical mechanism is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. There is no known animal condition corresponding to the inherited condition of depression in humans, but various procedures have been described that produce in animals behavioural states typical of human depression.

In the field of anxiety research, animal models are used as screening tools in the search for compounds with

therapeutic potential and as simulations for research on mechanisms underlying emotional behaviour. However, a solely pharmacological approach to the validation of such tests has resulted in distinct problems with their applicability to systems other than those involving the benzodiazepine/GABA_A receptor complex.

More than 30 animal models of anxiety are currently in use and, while some are based on physiological (e.g., hyperthermia) or endocrine (e.g., plasma corticosterone) responses to stress, the vast majority are behavioural in nature. Behavioural models may conveniently be classified as either conditioned or unconditioned responses to stimuli which appear capable of causing anxiety in humans.

The reactive oxygen species (ROS) and free radicals are by products of biological metabolism, which is responsible for cell membrane breakdown, membrane protein damage and DNA mutation. The Cadamba is a medicinal plant known to have antioxidant properties that are found particularly in its leaves.

As per literature review antioxidant properties in the methanolic extract of the Cadamba leaves were assayed by estimating liver and kidney tissue enzymes using the 2'-diphenyl-1-picrylhydrazyl (DPPH) assay, the superoxide anion radical scavenging assay and DNA damage. It was found that the Cadamba possesses potent antioxidant properties. Further, UPLC-ESI-QTOF/MS has also confirmed the presence of various bioactive compounds from the Cadamba leaves having antioxidant properties. As per literature review Cadamba showed significant increase in ketamine induced sleeping time. It also exhibited significant increase ($P < 0.05$, 0.01 and 0.001) in latency to clonic convulsion, tonic extension and time of death in PTZ and INH models at all tested doses, whereas in the MES model, the lower dose was found to be effective when compared with the higher doses (200 and 400 mg/kg, p.o.).

In another study as per literature review result was obtained from Yeast induced pyrexia method indicated that Chloroform, ethanol, distilled water extracts have significant onset of action as reduction of temperature by these extracts was found within 30 minutes. Whereas the reduction of temperatures with Petroleum ether and Solvent ether extracts was late. In all extracts the temperatures were reduced to normal till 180 minutes. Even Paracetamol also has significantly reduced rectal temperature to the extent of 37.70°C from 30 min. to 180 minutes. All the results were compared with Control group. All previous studies show that Cadamba is

having the activities related to CNS. It is having minerals, vitamins and amino acids as a chemical constituent which already have proved activity to cure the stress related disorders especially anxiety, depression and insomnia.

After selection of *Neolamarckia cadamba*, acute oral toxicity was detected with different extracts (ENC & AQNC) having dose (5, 50, 300, 1000 mg/kg) via oral route, shows no change in behavioral responses and observation shows no acute oral toxicity. Hence depending upon it, Dose was selected 250 mg/kg & 500 mg/kg for our experimental work. Phytochemical screening of *Neolamarckia cadamba* showed their presence of following contents in different part of the plants.

Anti-Anxiety Model

Neolamarckia cadamba (250 mg/kg & 500 mg/kg) aqueous and ethanolic extract were evaluated in swiss albino mice by elevated plus-maze (EPM). The effect of diazepam (DZP; 1 mg/kg) was also assessed.

In EPM (Elevated plus maze), when group - Control 1% w/v sodium CMC (5mg/kg, p.o) drug administration have shown their time spent in open arms (60.2 ± 2.854) and number of crossings (4.6 ± 0.376) insignificant. When aqueous extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown time spent in open arms (210.2 ± 2.374) and number of crossings (12.2 ± 0.384) is significant. When aqueous extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown time spent in open arms (262.4 ± 1.664) significant and number of crossings (13.6 ± 0.512) is significant.

When Ethanolic extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown time spent in open arms (198.0 ± 2.776) and number of crossings (12.0 ± 0.448) is significant. When Ethanolic extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown time spent in open arms (270.8 ± 2.598) and number of crossings (12.6 ± 0.506) is significant. When standard dose of Diazepam (1mg/kg) was orally administered, have shown time spent in open arms (290 ± 1.818) and number of jumps (16.6 ± 0.812) is more significant.

Antidepressant model

Neolamarckia cadamba (250 mg/kg & 500 mg/kg) Aqueous and ethanol extract was evaluated in swiss albino mice in Tail Suspension test (TST). The effects of fluoxetine (FXT; 10 mg/kg) were also being assessed.

In TST (Tail suspension test), Group - Control 1% w/v sodium CMC (5mg/kg, p.o) drug administration have shown their immobility (255 ± 4.806) insignificant. When

aqueous extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown immobility (90.2 ± 2.384) is significant. When aqueous extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown immobility (42.4 ± 1.855) is significant.

When Ethanolic extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown immobility (79 ± 2.764) is significant. When Ethanolic extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown immobility (38.8 ± 2.696) is significant. When standard dose of fluoxetine (10 mg/kg) was orally administered, have shown immobility (16.2 ± 1.626) is more significant.

Biochemical estimation

In Estimation of plasma corticosterone levels it is known that stress enhances the activity of the hypothalamus-pituitary-adrenal (HPA) axis and results in increased secretion of corticosteroids from the adrenal cortex. Cortisol and corticosterone are thus often used as biomarkers for stress and depressive disorders. Although corticosterone is considered the main glucocorticoid involved in regulation of stress responses in rodents, researchers often choose to detect cortisol for stress indicators in consideration of convenience and kits availability.

Group - Control 1% w/v sodium CMC (5mg/kg, p.o) drug administration have shown their corticosterone level is (198.87 ± 0.58) insignificant. When aqueous extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown corticosterone level (176.48 ± 1.46) also insignificant. When aqueous extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown corticosterone level (170.98 ± 2.26) is significant.

When Ethanolic extract of *Neolamarckia cadamba* (250 mg/kg) orally administered, have shown corticosterone level (168.16 ± 1.27) is significant. When Ethanolic extract of *Neolamarckia cadamba* (500 mg/kg) orally administered, have shown corticosterone level (158.48 ± 0.30) is significant. When standard dose of fluoxetine (10 mg/kg) was orally administered, have shown corticosterone level (140.82 ± 0.36) is more significant.

Conclusion

Neolamarckia cadamba ethanolic and aqueous extracts were tested in swiss albino mice at dosages of 250 and 500 mg/kg. *Neolamarckia cadamba* has anxiolytic and antidepressant properties, likely via BZD receptors, selective serotonin reuptake inhibitors. Oral

administration of *Neolamarckia cadamba* (250 mg/kg) showed considerable immobility (90.22.384). Oral administration of *Neolamarckia cadamba* (500 mg/kg) showed significant immobility (42.41.855). Oral administration of *Neolamarckia cadamba* ethanolic extract (250 mg/kg) increased immobility (792.764). Oral administration of *Neolamarckia cadamba* (500 mg/kg) showed considerable immobility (38.82.696). Oral administration of fluoxetine (10 mg/kg) increased immobility (16.21.626). It also encourages further investigation into the anxiolytic and depressive characteristics of *Neolamarckia cadamba*. This plant's potent pharmacological qualities may be utilised to treat a variety of anxiety and depression problems, but only in clinical investigations.

Disclosure Statement

There are no conflicts of interest.

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