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Evaluation of the Anxiolytic and Antidepressant Activities of Hydrastis canadensis, Nux moschata and Anacardium orientale Mother Tinctures in Swiss Albino Mice

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Abstract

Objective: the objective of the present study was to assess the antianxiety and antidepressant activity of homeopathic tinctures (*Hydrastis Canadensis*, *Nuxmoschata* and *Anacardium orientale*) in animal model of anxiety and depression.

Method: Plant origin tinctures were collected from local homeopathic pharmacy. The tinctures were subjected to preliminary phytochemical analysis. In vivo anti-anxiety activity and anti-depressant activity evaluated by dark and light exploration test, Elevated plus maze, forced swim test and locomotor activity respectively. Mice were divided into 6 groups (n=6 each). Group 1 control received normal saline 10ml/kg p.o. Group II test group received *Hydrastis* tincture 0.5µl p.o, group III test group received Nuxmoschata tincture 0.5µl p.o, group IV test group received Anacardium orientale tincture 0.5µl p.o, Group V receives combination of these three tincture 0.5µl p.o and group VI standard group receives Escitalopram 20 mg/kg p.o., respectively after treatment each group check the behaviour of animals.

Results: The preliminary phytochemical examination showed the presence of flavonoids alkaloids, glycosides, terpenoids and carbohydrates. These tinctures showing significant antianxiety and antidepressant activity in mice model. In anxiety dark and light exploration and elevated plus maze method both showing tincture treated group possess significant increase in exploration light compartment and open arms of EPM ie, increase in antianxiety activity. In forced swim test each tincture and combination of tincture possess decrease immobility time compared to control. In locomotor activity increase in locomotion of mice treated by tinctures compared to control. So this tinctures also showing antidepressant activity.

Conclusion: the results obtain from the study indicate that these homeopathic tinctures (*Hydrastis Canadensis*, Nuxmoschata and *Anacardium orietale*) possess both antianxiety and antidepressant activity. The activity might be due to presence of the phytoconstituents including flavonoids (keampferol and quarcetin), alkaloid (berberine), anacardic acids, glycosides and carbohydrates in the tinctures. Still, the determination of biogenic amine is recommended so that we could correlate them with their pharmacological activity

Keywords: Antianxiety activity; Antidepressant activity; *Hydrastis Canadensis*; *Nuxmoschata*; *Anacardium orientale;* Tincture.

1.Introduction

Among the most common neuropsychiatric conditions affecting human health globally, anxiety and depression are linked to a substantial social, professional, and financial burden. Excessive fear, apprehension, autonomic hyperactivity, and behavioural abnormalities are the hallmarks of anxiety disorders, while persistent sadness, lack of interest, sleep difficulties, diminished concentration, and suicidal thoughts are frequently linked to depression. Millions of people worldwide suffer from these mental illnesses, according to the World Health Organization, making them a serious public health issue [(1)].

While a number of synthetic medications, including benzodiazepines, tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (SSRIs), are currently available to treat anxiety and depression, long-term use of these medications is frequently linked to side effects, such as sedation, dependence, cognitive impairment, tolerance, gastrointestinal problems, and withdrawal symptoms [(2, 3)]. Therefore, in recent years, there has been a growing focus on finding safer and more effective alternative medicines.

Numerous neurotransmitters, neuroendocrine pathways, inflammatory mediators, and oxidative stress processes interact intricately in the pathophysiology of anxiety and depression. The development of these illnesses is thought to be significantly influenced by changes in the function of the γ-aminobutyric acid (GABA), serotonin (5-HT), norepinephrine, dopamine, glutamate, and hypothalamic-pituitary-adrenal (HPA) axis [(4,5)]. Additionally, oxidative stress and neuroinflammation have been shown in experiments to exacerbate neuronal malfunction, which can result in aberrant behaviour linked to anxiety and depression [(6)].

Due to their varied phytochemical components and generally superior safety profile, medicinal plants and traditional medical systems have been thoroughly investigated for the treatment of illnesses of the central nervous system. One complementary medical method that uses highly diluted formulations obtained from plants, minerals,

and animals for medicinal purposes is homoeopathy. Although a number of homoeopathic mother tinctures have long been used to treat neurological and psychological disorders, there is still no empirical proof of their pharmacological effects [7].Among various homeopathic remedies, Hydrastis canadensis, Nuxmoschata, and Anacardium orientale have attracted attention due to their traditional therapeutic applications. Hydrastis canadensis (goldenseal) contains bioactive alkaloids such as berberine and hydrastine, which possess neuroprotective, antioxidant, and anti-inflammatory properties [8]. Nuxmoschata derived from Myristica fragrans contains flavonoids, essential oils, and phenolic compounds reported to exhibit central nervous system modulatory activity including sedative and anxiolytic effects [9].Anacardium orientale, which is made from Semecarpus anacardium, is traditionally used to treat mental disorders, memory loss, and nerve debility. It includes biologically active substances such flavonoids and anacardic acids that have neuroprotective and antioxidant properties [10].

Preclinical behavioural models play a crucial role in the screening and evaluation of potential anxiolytic and antidepressant agents. Experimental paradigms such as the Forced Swim Test (FST) are widely employed due to their reliability and predictive validity in assessing behavioural responses associated with depression and antidepressant activity [11]. Similarly, behavioural models such as the Elevated Plus Maze (EPM) and Light–Dark Exploration Test are extensively used for evaluating anxiety-related responses and the efficacy of anxiolytic agents [12]. These models enable the identification of neuropharmacological effects of test substances and provide valuable insights into their potential therapeutic utility. Furthermore, stress-induced behavioural alterations in rodents closely resemble certain features of human anxiety and depressive disorders, making them useful tools for investigating novel therapeutic interventions and exploring the pharmacological properties of plant-derived and traditional medicinal preparations [13].

In addition to the Forced Swim Test, locomotor activity assessment is frequently employed in preclinical studies to further evaluate antidepressant-like effects and general behavioural responses. Locomotor activity reflects the functional status of the central nervous system and is influenced by neurotransmitters such as dopamine, serotonin, and norepinephrine, which play crucial roles in mood regulation and motivation [14]. Antidepressant agents often enhance exploratory behaviour and spontaneous motor activity by modulating these neurotransmitter systems. Therefore, assessment of locomotor activity provides supportive evidence for antidepressant potential and helps distinguish genuine antidepressant effects from nonspecific behavioural suppression or sedation induced by test substances [15].

Anxiety-related behaviours in experimental animals are commonly evaluated using behavioural paradigms based on the natural conflict between exploratory drive and fear of unfamiliar environments. The Light–Dark Exploration Test is one of the most widely used models for assessing anxiolytic activity, as rodents naturally prefer dark and protected environments while also exhibiting an innate tendency to explore novel surroundings. Anxiolytic agents generally increase the time spent in the illuminated compartment and the frequency of transitions between the light and dark compartments, reflecting a reduction in anxiety-related behaviour. Therefore, this model serves as a reliable and sensitive tool for the preliminary screening of compounds with potential anxiolytic properties [16].

The Elevated Plus Maze (EPM) is another well-established and validated behavioural model for evaluating anxiety in rodents. The test is based on the natural aversion of rodents to open and elevated spaces and their spontaneous tendency to explore novel environments. An increase in the number of entries into the open arms and the duration of time spent in the open arms is considered indicative of anxiolytic activity, whereas avoidance of the open arms reflects anxiety-like behaviour. Due to its simplicity, sensitivity, and predictive validity, the Elevated Plus Maze is extensively employed in the preclinical assessment of anxiolytic agents and in the investigation of neuropharmacological mechanisms involved in anxiety disorders [17].

2. Materials and Methods

2.1 Collection and Authentication of Homeopathic Tinctures

The Hydrastis canadensis, Nuxmoschata, and Anacardium orientale homeopathic mother tinctures were purchased from a nearby homeopathic pharmacy in the Malappuram district of Kerala, India. Dr. Alikutty V.V., BHMS (Reg. No. 4095/A), a qualified homeopathic practitioner, verified the authenticity of the tinctures. Until they were needed again, the tinctures were kept in the prescribed storage conditions .

2.2 Preliminary Phytochemical Screening

Standard qualitative chemical assays were used to do a preliminary phytochemical screening of the chosen tinctures in order to identify alkaloids, flavonoids, phenols, tannins, glycosides, carbohydrates, proteins, amino acids, steroids, and terpenoids. Alkaloids were tested using the Mayer, Wagner, and Dragendroff ‘s methods. Carbohydrates were tested using Molisch's, Benedict's, and Fehling's methods; phenolic compounds and flavonoids were tested using ferric chloride and alkaline reagents. For steroidal and terpenoid substances, Liebermann-Burchard and Salkowski tests were employed [18,19].

2.3 In-vivo pharmacological evaluation

2.3.1 Animals

Swiss albino mice (20-25g) of either sex were procured from the Devaki Amma Memorial College of Pharmacy, Kerala, India. All animals were maintained in an animal housing facility of institute and provided a standard laboratory diet and water ad libitum; animals were maintained at ambient temperature (22±2°C), at a relative humidity (55±2°C), and with a 12 h light–dark cycle. The Institutional Animal Ethics Committee approved the

experimental protocol **(Ethical Approval No: DAMCOP/IAEC/070)**. During exposure regimens, animals were only given freshly prepared solutions of drugs or chemicals [20].

2.3.2 Experimental Protocol

On the first day of experiment the animals were divided randomly into six groups of six animals each. Anxiety was induced by low temperature-induced stress for the I, II, III, IV, V and VI groups and continued for 10 days. Control animals were distilled water and standard group Escitalopram given orally by using intragastric catheter, tincture given orally by micropipette. The last dose was given 30 min prior to behavioural testing.

2.3.2.1 Animal grouping

- Group I : Control group (Normal saline 1ml, p.o)
- Group II : Test group (*H. canadensis* tincture 0.5 µl, p.o).
- Group III : Test group (Nuxmoschata tincture 0.5µl, p.o).
- Group IV : Test group (*Anacardium orientale* tincture 0.5µl, p.o).
- Group V : Test group (Combination of *H. canadensis*, Nuxmoschata and *A.orientale* 0.5µl, p.o*)*
- Group VI : Standard drug (Escitalopram 20mg/kg, p.o).

2.3.2.2 Antidepressant study

- Forced swim test.
- Locomotor activity.

2.3.2.3 Antianxiety study

- Dark and light exploration test.
- Elevated plus maze.

2.3.3 Animal models of antidepressant study

Antidepressant activity was indicated the mood elevating due to various mechanisms of the antidepressant drugs, such as inhibition of the enzyme of monoamine oxidase, inhibition of reuptake bio amines and enhancement of the concentration of 5-HT. Later on, inhibition of reuptake of bio amines was found to be main mechanism of action to down regulation of β receptor. Several lines of preclinical and clinical evidence indicates that enhancement of 5-HT mediated neurotransmission might underline the therapeutic effect of most of the antidepressant. This behavioural effect very similar to that found by other author after treating mice with classical antidepressant drugs as IMI various models are like,

2.3.4 Forced swimming Test (FST)

Experimental Design: Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into six groups of six animals each,

- Group I : Control group (Normal saline 1ml, p.o)
- Group II : Test group (*H. canadensis* tincture 0.5µl, p.o).
- Group III : Test group (Nuxmoschata tincture 0.5µl, p.o).
- Group IV : Test group (*Anacardium orientale* tincture 0.5µl, p.o).
- Group V :Test group (Combination of *H. canadensis*, *Nuxmoschata* and *A.orientale* 0.5µl, p.o*)*
- Group VI : Standard group (Escitalopram 20 mg/kg p.o).

They were brought to the laboratory at least one day before the experiment and were housed separately in cages with free access to food and water. This test was sensitive to all major classes of antidepressant drugs, hence used as an animal model of depression like behaviour. The animals were made to swim individually in an open cylindrical container, containing 15cm of water at 25˚C, for a test period of 6 minutes. After a brief period of vigorous activity for about two minutes, mice maintain a typical immobile posture. The animals were considered immobile when they float in an upright position, making only negligible movements to maintain their head above water. The total duration of immobility was recorded and changes in the same were studied for various treatment groups [21].

2.3.5 Locomotor activity

Experimental Design: Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into six groups of six animals each, the test has been proposed for selective identification of Antidepressant drugs.

- Group I : Control group (Normal saline 1ml, p.o)
- Group II : Test group (*H. canadensis* tincture 0.5µl, p.o).
- Group III : Test group (Nuxmoschata tincture 0.5µl, p.o).
- Group IV : Test group (*Anacardium orientale* tincture 0.5µl, p.o).
- Group V : Test group (Combination of *H. canadensis, Nuxmoschata and A.orientale* 0.5µl, p.o*)*
- Group VI : Standard group (Escitalopram 20mg/kg, p.o).

After the treatment of standard and test, the animal was placed the center of square arena of Infrared Actimeter for ten minutes and locomotor activity was recorded. The number of crossing light beams by the mice was record as the locomotor activity. The animals were acclimatized before commencement of experiments [22, 23].

2.3.6 Animal models for antianxiety study

The animal model was considered one of most widely validated tests of assaying sedative and anxiolytic substances such as benzodiazepines. The test drug induced anxiolytic effect beginning at lower doses employed. An increase of most important variables of EPM test was found as follow; the percentage of time of mice spend on the open arms as well as the percentage of entries in the dark arms. The anxiolytic effect was also evidenced through light and dark test. As with the EPM test, this model was useful for modelling of anxiety. The low dose dependent effect could be attributed to biological variability, as well as chemical .

2.3.7 Light-Dark model

Experimental Design Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into six groups of 6 animals each

- Group I : Control group (Normal saline 1ml, p.o)
- Group II : Test group (*H. canadensis* tincture 0.5µl, p.o).
- Group III : Test group (Nuxmoschata tincture 0.5µl, p.o).
- Group IV : Test group (*Anacardium orientale* tincture 0.5µl, p.o).
- Group V : Test group (Combination of *H. canadensis, Nuxmoschata and A.orientale* 0.5µl, p.o*)*
- Group VI : Standard group (Escitalopram 20mg/kg, p.o).

The apparatus consists of a Plexiglas box with two compartments (20 x 20 cm) one of which was illuminated with a white light while the other remained dark. Each animal was placed at the centred of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space is recorded for 5 min. The animals were acclimatized before commencement of experiments [(24)].

2.3.8 Elevated Plus-Maze Test (EPM)

Experimental Design Swiss albino mice weighed around 20-25g were used for the study. Mice were divided into six groups of 6 animals each,

- Group I : Control group (Normal saline 1ml, p.o)
- Group II : Test group (*H. canadensis* tincture 0.5 µl, p.o).
- Group III: Test group (Nuxmoschata tincture 0.5 µl, p.o).
- Group IV: Test group (*Anacardium orientale* tincture 0.5 µl, p.o).
- Group V : Test group (Combination of *H. canadensis, Nuxmoschata and A.orientale* 0.5 µl, p.o*)*
- Group VI : Standard group (Escitalopram 20mg/kg, p.o).

The plus maze for mice consisted of two perpendicular open arms (30×5cm) and two closed arms (30×5×15cm) also in perpendicular position. The open and closed arms were connected with each other by a central platform (5×5cm). The lateral walls of the closed arm and floor of each arm were made of wood. The platform and lateral walls were painted in black. The maze was elevated at a height of 45cm above the floor. In the test-session, 30 minutes after the treatment with standard and sample, the animal was placed at the centre of the maze with its nose facing in the direction of one of the closed arms. The duration of the test period was 5 minutes, during which the following parameters were evaluated

- The number of entries into the open arm.
- The number of entries into the closed arm.
- Time spent in the open arm.
- Time spent in the closed arm.

Each animal was placed at the centred of the illuminated compartments; facing one of the dark places, as well as the number of entries in each space was recorded for 5 min [(25,26)].

2.3.9 Statistical Analysis

All the data were expressed as the mean ± standard error of the mean (SEM). The statistical significance of the difference between the groups was analysed by using Graph pad 6.0 software by applying one was analysis of variance (ANOVA) followed by Dunnett's test to make statistical comparison between various treatment groups. The results were consider statistically significant at $p<0.05$ level.

3. Results

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of *Hydrastis canadensis*, *Nuxmoschata*, and *Anacardium orientale* tinctures revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoids, and phenolic compounds. However, saponins, tannins, proteins, and amino acids were absent. The presence of flavonoids and alkaloids may contribute to the observed neuropharmacological activities.

Table no.1: Qualitative phytochemical screening of *Hydrastis canadensis, Nuxmoschata* and *Anacardium orientale* tincture

Sl. No.	Chemical constituents	Chemical test	Result
1	Alkaloids	Dragendroff's test	Positive
		Wagner's test	Negative
		Mayer's test	Positive
		Hager's test	Positive
2	Carbohydrates	Benedict's test	Positive
		Molisch's test	Positive
		Fehling's test	Positive
		Barfoed's test	Positive
3	Glycosides	Anthraquinone glycoside	Negative
		Test for cardiac glycoside	Positive

4	Saponins	Foam test	Negative
5	Flavonoids	Sodium hydroxide test	Positive
		Ferric chloride	Positive
6	Protein and Amino acids	Ninhydrin test	Negative
		Milon's test	Negative
		Biuret test	Negative
7	Steroids and Terpenoids	Libermann and Burchard test	Positive
		Salkowski test	Positive
8	Tannins	Ferric chloride test	Negative
		Gelatin test	Negative
9	Phenols	Ferric chloride test	Positive

(Positive) indicate the presence of active constituents & (Negative) indicates the absence of active constituents

3.2 Antidepressant Activity

3.2.1 Forced Swim Test

The antidepressant activity of the tinctures was evaluated using the Forced Swim Test. Administration of *Hydrastis canadensis*, *Nuxmoschata*, and *Anacardium orientale* tinctures significantly reduced the duration of immobility when compared with the control group (**p<0.01). The combination-treated group exhibited marked reduction in immobility time (62.08 ± 0.08 sec), which was comparable to the standard drug escitalopram (54.09 ± 1.41 sec).

The reduction in immobility time observed in the treated groups indicates significant antidepressant-like activity. Among the individual tinctures, *Anacardium orientale* showed better activity compared to other tinctures. The combination treatment demonstrated synergistic antidepressant activity.

Table no.2: Effect of tinctures on duration of immobility time in forced swim test

Sl No.	**Group**	**Duration of immobility**
`1	Group I : Control (Normal saline 10ml/kg p.o.)	119.71± 2.03
2	Group II : Test group (*Hydrastis canadensis* Tincture 0.5 µl p.o.)	85.01 ±0.80**
3	Group III : Test group (*Nuxmoschata* tincture 0.5 µl p.o)	83.18 ±1.24**
4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	74.46 ±1.24**
5	Group V : Test group (Combination of *H.canadensis*, *Nuxmoschata* and *A. orientale,* 0.5 µl p.o*)*	62.08 ± 0.08 **
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	54.09 ± 1.41**

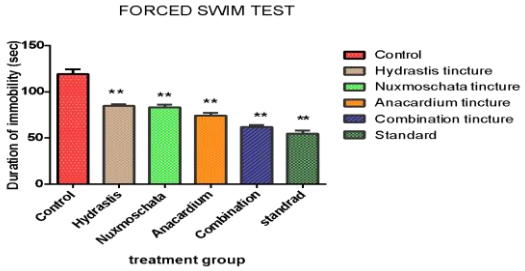


Fig. 1: Antidepressant activity by forced swim test

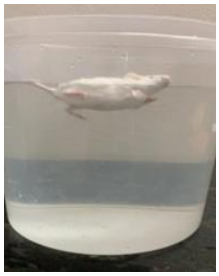


Fig. 2 struggling stage of mice



Fig. 3 Immobility stage of mice

A significant reduction in immobility period was observed in all groups when *Hydrastis canadensis* tincture, *Nuxmoschata* tincture and *Anacardium orientale* tincture were administered. Combination of tincture (**p<0.01) shows potent antidepressant activity while compared with that of control group, also combination showed synergistic effect when given alone.

Table no.3: Effect of tinctures on number of light beam crossed in locomotor activity

Sl.NO.	Group	Number of Light Beam Crossed
1	Group I : Control (Normal saline 10ml/kg p.o.)	834.16±1.15
2	Group II : Test group (*Hydrastis canadensis* Tincture 0.5 µl p.o.)	1070.66± 2.021**
3	Group III: Test group (*Nuxmoschata* tincture 0.5 µl p.o)	1108.83±3.75**
4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	1174.66± 3.175**
5	Group V : Test group (Combination of *H.canadensis*, *Nuxmoschata* and *A. orientale,* 0.5µl p.o*)*	1406.83± 4.23**
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	1515.16± 5.48**

Values are expressed as mean ± SEM. One way ANOVA comparison between treatment groups with control (Dunnet's test). The data are considered significant as **p<0.01.

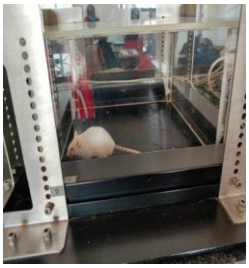


Fig.4: Depressed stage of mice

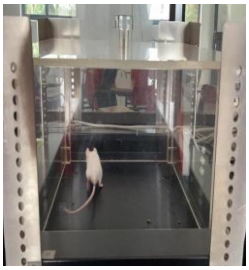


Fig.5: locomotion stage of mice

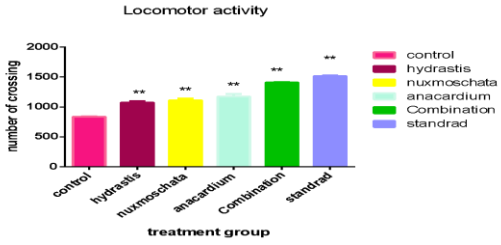


Fig. 6: Antidepressant activity by locomotor test

The effects of tinctures on locomotor activity were studied and a significant increase in the antidepressant activity in mice was observed. *Hydrastis Canadensis* tincture, *Nuxmoschata* tincture and *Anacardium orientale* tincture has shown an increase in the locomotor activity. Hence, the combination of three tinctures has shown an increase in the locomotor activity while compared with that of the control group

3.4 Antianxiety study

3.4.1 Dark & light exploration test

Table no.4: Effect of tinctures on time spent in light box in dark and light exploration test

S. NO	GROUP	Time spent(sec)
1	Group I : Control (Normal saline 10ml/kg, p.o.)	6.35± 0.53
2	Group II : Test group (*Hydrastis canadensis* Tincture 0.5 µl p.o.)	41.22± 0.35**
3	Group III Test group (*Nuxmoschata* tincture 0.5 µl p.o)	20.09± 0.60**

4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	41.05± 0.32**
5	Group V : Test group (Combination of *H.canadensis Nuxmoschata* and *A. orientale,* 0.5 µl p.o*)*	47.12± 0.10 **
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	66.13± 1.9**

Values are expressed as mean ± SEM. One way ANOVA comparison between treatment groups with control (Dunnet's test). The data are considered significant as **p<0.01.



Fig.7: Time spend at light box exploration test (time spend in light box)

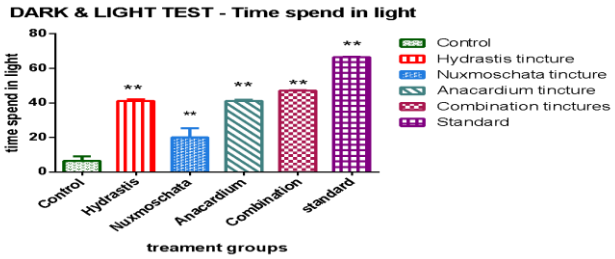


Fig no.8 Anti-anxiety activity by dark and light

Effect of tinctures in the dark and light exploration study, an increase in time spent at light box was observed. This showed a significant anxiolytic activity in animal models. *Hydrastis canadensis* tincture (**p<0.01) and *Anacardium orientale tincture* (**p<0.01) was showed significant anxiolytic activity when compared to the control group. *Nuxmoschata* tincture (**p<0.01) showed less activity when compared to control. Combination of these tinctures exhibited significant (**p<0.01) anxiolytic activity when comparison with the control group.

Table no.5: Effect of tinctures on number of crossing between dark and light box in dark and light exploration test

SL NO	**Group**	**Number of crossing b/w dark and light box**
1	Group I : Control (Normal saline 10ml/kg p.o.)	5.83 ± 0.05
2	Group II: Test group (*Hydrastis canadensis* tincture 0.5µl p.o.)	11.17±0.19**
3	Group III : Test group (Nuxmoschata tincture 0.5 p.o)	11.32± 0.13**
4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	10.00±0.177**
5	Group V : Test group (Combination of *H.canadensis*, Nuxmoschata and *A. orientale,* 0.5 µl p.o*)*	12.83±0.23**
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	17.05 ±0.32**

Values are expressed as mean ± SEM. One way ANOVA comparison between treatment groups with control (Dunnet's test). The data are considered significant as **p<0.01



Fig.9 Crossing between dark and light box test (time spend in light box)

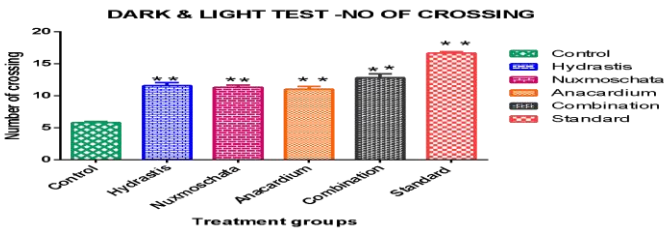


Fig no.10 Anti-anxiety activity by dark and light exploration

Effect of tinctures in the study of dark and light exploration study, an increase in crossing between light and dark box were observed. This showed significant anxiolytic activity in the animal model. Both *Hydrastis canadensis* tincture (**p<0.01) and *Nuxmoschata* tincture (**p<0.01) alone showed anxiolytic activity when compared to

control and *Anacardium orientale* (**p<0.01) alone showed less activity when compared to control. Combination of these tinctures showed significant anxiolytic activity observed in comparison with the control group.

3.4.2 Elevated plus maze

Table no.6: Effect of tinctures on time spend in open arm in Elevated plus maze test

SL NO	Group	Time spent in Open arm (sec)
1	Group I : Control (Normal saline 10ml/kg p.o.)	11.26 ± 0.06
2	Group II : Test group (*Hydrastis canadensis* tincture 0.5 µl p. o.)	21.08 ± 0.14**
3	Group III : Test group (Nuxmoschata tincture 0.5 µl p.o)	36.24 ± 0.09**
4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	71.20 ± 0.06**
5	Group V : Test group (Combination of *H.canadensis*, Nuxmoschata and *A. orientale,* 0.5 µl p.o*)*	96.96 ± 0.13**
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	111.15± 0.04**

Values are expressed as mean ± SEM. One way ANOVA comparison between treatment groups with control (Dunnet's test). The data are considered significant as **p<0.01.



Fig 11: Explore in Enclosed arm



Fig.12: Explore in open arm

When considering the effect of tinctures in elevated plus maze method, each group exhibited significant increase in the time exploring in the open arm, when compared to control. In *Hydrastis canadensis*, the tincture showed a significant (**p<0.01) increase in the time spent at open arm. Both *Nuxmoschata tincture* (**p<0.01) and *Anacardium orientale* tinctures showed significant (**p<0.01) increase in the exploring open arm, in combination with these tinctures showed potent significant (**p<0.01) increase in time spend open arm.

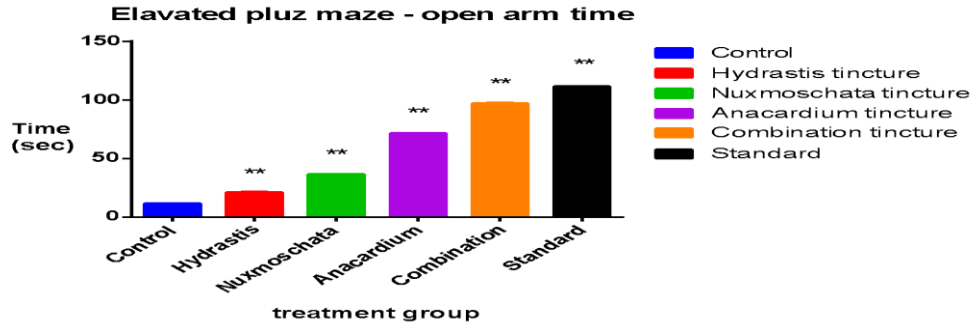


Fig 13: Anti-anxiety activity by Elevated plus maze (Open arm time)

Table no.7: Effect of tinctures on number of entry to open arm in elevated plus maze test

SL NO	Group	Number of entry to Open arm
1	Group I : Control (Normal saline 10ml/kg p.o)	1.50 ± 0.34
2	Group II : Test group (*Hydrastis canadensis* tincture 0.5 µl p.o.)	3.61 ± 0.30^{ns}

3	Group III : Test group (Nuxmoschata tincture 0.5 µl p.o)	5.33 ± 0.16**
4	Group IV : Test group (*Anacardium orientale* tincture 0.5 µl p.o)	25.21 ± 0.30**
5	Group V : Test group (Combination of *H.canadensis*, Nuxmoschata and *A. orientale,* 0.5 µl p.o*)*	34.10 ± 0.48**
6	Group VI : Standard drug (Escitalopram 20 mg/kg)	43.33 ± 0.42**

Values are expressed as mean ± SEM. One way ANOVA comparison between treatment groups with control (Dunnet's test). The data are considered significant as **p<0.01and non-significant as **P<0.05.



Fig.14: Explore in Enclosed arm

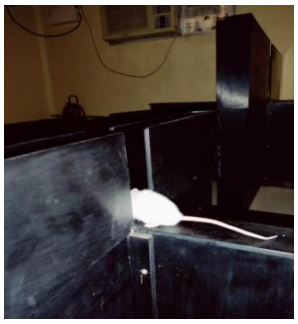


Fig.15: Explore in open arm

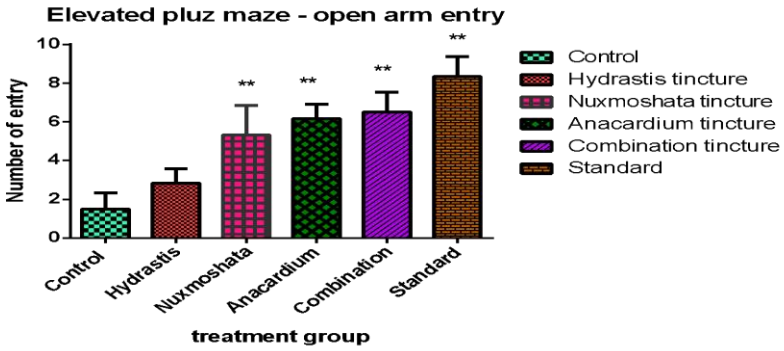


Fig 16: Anti-anxiety activity by elevated plus maze (Open arm entry)

In the effect of tinctures in elevated plus maze method, each group showed a significant increase in the number of crossing between open arm and closed arm when compared to control. In *Hydrastis canadensis* tincture showed non-significant (^{ns} p<0.05) increase in the number of crossings between open arm and closed arm. Both Nuxmoschata tincture and *Anacardium orientale* (**p<0.01) tincture showed significant increase in the number of crossings between open arm and closed arm whereas, in combination of these tinctures showed potent effect when compared to the control group.

4. Discussion

Depression is a prevalent psychiatric disorder and a major contributor to the global disease burden. Although several antidepressants are available for its treatment, their use is often limited by delayed onset of action and adverse effects. Consequently, there is growing interest in alternative therapeutic approaches, including homeopathic medicines. Several plant-derived compounds have demonstrated antidepressant activity through modulation of neurotransmitter systems and neuronal signaling pathways.

The Forced Swim Test (FST) is a widely accepted experimental model for screening antidepressant activity. In the present study, antidepressant activity of *Hydrastis canadensis*, *Nux moschata*, and *Anacardium orientale* tinctures was evaluated using the FST by measuring immobility time, an indicator of behavioral despair. After the treatment, the tinctures increased swimming and struggling behavior while reducing immobility time, indicating antidepressant-like activity.

Among the tested preparations, *Hydrastis canadensis* significantly (**p<0.01) increased struggling time, which may be attributed to the presence of berberine alkaloids known to modulate biogenic amines such as norepinephrine (NE) and serotonin (5-HT). *Anacardium orientale* also significantly (**p<0.01) increased struggling time, possibly due to its flavonoid and phenolic constituents that influence catecholamine levels. The combination of all three tinctures significantly (**p<0.01) reduced immobility time compared to the control group, although its effect was less pronounced than that of the standard drug, escitalopram [(27)].

Locomotor activity testing further supported the antidepressant potential of the tinctures. All treatment groups exhibited reduced immobility and enhanced exploratory behavior compared to the control group. *Hydrastis canadensis*, *Nux moschata*, and *Anacardium orientale* each demonstrated antidepressant-like effects, while the combination treatment significantly (**p<0.01) reduced immobility time, though it remained less potent than

escitalopram. These effects may be associated with increased levels of dopamine, acetylcholine, and other monoaminergic neurotransmitters involved in motor and behavioral regulation [28, 29].

Anxiety commonly accompanies depressive disorders, and currently available anxiolytic agents are associated with several adverse effects. Therefore, the search for safer alternatives remains important. The anxiolytic activity of the tinctures was evaluated using the Light–Dark Exploration Test and Elevated Plus Maze (EPM).

In the Light–Dark Exploration Test, all tinctures increased the time spent in the illuminated compartment and the number of transitions between compartments compared to the control group, indicating anxiolytic activity. *Hydrastis canadensis* and *Anacardium orientale* significantly (**p<0.01) increased these parameters, whereas *Nux moschata* produced comparatively weaker effects. The combination treatment significantly (**p<0.01) enhanced time spent in the light compartment and the number of crossings, suggesting potent anxiolytic activity. These effects may be related to modulation of monoaminergic neurotransmission and GABAergic mechanisms mediated by flavonoid constituents [30].

The EPM findings further confirmed the anxiolytic potential of the tinctures. Treatment increased both the number of entries into the open arms and the time spent in the open arms compared to the control group. *Nux moschata* and *Anacardium orientale* produced significant (**p<0.01) increases in these parameters, whereas *Hydrastis canadensis* showed a non-significant improvement. The anxiolytic effects of *Nux moschata* and *Anacardium orientale* may be attributed to flavonoids such as quercetin and kaempferol, and bioactive compounds including anacardic acid, which may interact with GABAergic pathways similarly to benzodiazepines [31].

The observed antidepressant and anxiolytic activities may be related to modulation of monoaminergic and GABAergic neurotransmitter systems. Berberine present in *Hydrastis canadensis* has been reported to influence serotonin, norepinephrine, and dopamine levels, while flavonoids present in *Nux moschata* and *Anacardium orientale* are known to interact with GABAA receptors and enhance neurotransmission associated with mood regulation [32]. These findings are consistent with previous reports demonstrating the involvement of serotonergic, dopaminergic, and GABAergic pathways in depression and anxiety.

Overall, the present study demonstrates that *Hydrastis canadensis*, *Nux moschata*, and *Anacardium orientale*, particularly in combination, possess significant antidepressant and anxiolytic activities in experimental animal models. However, further studies are required to elucidate their precise mechanisms of action and to establish their clinical efficacy and safety.

5. Conclusion

The present study was design to evaluate Antianxiety and Antidepressant activty of homeopathic tinctures (*Hydrastis canadensis, Nuxmoschata* and *Anacardium orientale*) in Swiss albino mice. These tinctures were collected and authenticated by homeopathic physician.

The preliminary phytochemical analyses were carried out on each tincture, showed the presence of alkaloids, carbohydrates, flavonoids, glycosides and phenolic compounds.

The antidepressant and Antianxiety were evaluated in 36 Swiss albino mice in either sex divided into 6 groups each group contain 6 animals. Control, each tincture, combination each tincture and standard escitalopram taken as in specific group respectively.

The antidepressant activity was evaluated by Forced Swim Test (FST) and locomotor activity. Anti-anxiety study was evaluated by Dark and Light exploration test (D/L) and Elevated Plus Maze (EPM) method. The results showed each tincture possess significant Antianxiety and Antidepressant activity. From each tincture and the combination of tinctures, the combination of tincture was found to have potent significant activity as compare to each tincture and control in both Antianxiety and Antidepressant models.

From the results it can be concluded that the homeopathic tinctures (*Hydrastis canadensis, Nuxmoschata* and *Anacardium orientale*) possess promising Antianxiety and Antidepressant activities. The activity might be due the presence of the Phytoconstituents including flavonoids (keampferol, Quarcetin), Alkaloids (berberine), anacardic acid and carbohydrates in the tinctures. Possible mechanism in the antidepressant activity may be due antioxidant property of flavonoids and also they can increase the neurotransmitters especially biogenic amines serotonin, norepinephrine in brain. Possible mechanism of antianxiety activity GABAergic, the flavonoid had structural similarity to the benzodiazepine receptor which also enhancing GABAergic mechanism. Exact mechanism behind the activities could not be concluded at the point because of number of Phytoconstituents present in the tincture. These interesting activities require further research to identify and isolate the compounds involved followed by mechanism of action.

Reference

1. World Health Organization. Depression and other common mental disorders: global health estimates. Geneva: World Health Organization; 2017.
2. Baldwin DS, Aitchison K, Bateson A, Curran HV, Davies S, Leonard B, et al. Benzodiazepines: risks and benefits. A reconsideration. J Psychopharmacol. 2013;27(11):967-971. doi:10.1177/0269881113503509.
3. Ferguson JM. SSRI antidepressant medications: adverse effects and tolerability. Prim Care Companion J Clin Psychiatry. 2001;3(1):22-27. doi:10.4088/PCC.v03n0105.
4. Cui R. A systematic review of depression. Curr Neuropharmacol. 2015;13(4):480.
5. Bao AM, Swaab DF. The human hypothalamus in mood disorders: the HPA axis in the center. IBRO Rep. 2019;6:45-53. doi:10.1016/j.ibror.2018.11.008.

6. Santos P, Herrmann AP, Elisabetsky E, Piato A. Anxiolytic properties of compounds that counteract oxidative stress, neuroinflammation, and glutamatergic dysfunction: a review. Braz J Psychiatry. 2019;41(2):168-178. doi:10.1590/1516-4446-2018-0005.
7. Taschner G. Homeopathy and mental health care: integrative approaches in psychiatry and psychology. Complement Ther Clin Pract. 2018;31:165-171.
8. Yuan NN, Cai CZ, Wu MY, Su HX, Li M, Lu JH. Neuroprotective effects of berberine in animal models of Alzheimer's disease: a systematic review of pre-clinical studies. BMC Complement Altern Med. 2019;19:109. doi:10.1186/s12906-019-2510-z.
9. Perveen T, Khan NU, Abbas SQ, Tareen RB. Neuroprotective and CNS modulatory activities of Myristica fragrans: a review. Pak J Pharm Sci. 2018;31(5):2517-2523.
10. Raut SS, Bhosale UA, Kulkarni YA. Neuroprotective effect of Semecarpus anacardium Linn. in experimental neurological disorders: a review. Pharmacogn Rev. 2017;11(21):1-7
11. Brandwein C, Leenaars CHC, Becker L, Pfeiffer N, Iorgu AM, Hahn M, et al. A systematic mapping review of the evolution of the rat Forced Swim Test: Protocols and outcome parameters. *Pharmacol Res*. 2023;196:106917. doi:10.1016/j.phrs.2023.106917.
12. Rosso M, Wirz R, Loretan AV, Sutter NA, Pereira da Cunha CT, Jaric I, et al. Reliability of common mouse behavioural tests of anxiety: a systematic review and meta-analysis on the effects of anxiolytics. *Neurosci Biobehav Rev*. 2022;143:104928. doi:10.1016/j.neubiorev.2022.104928.
13. Desingu R, Sadasivam B, Kalra SS, Lakhawat B. Animal models of anxiety: a review. *Int J Basic Clin Pharmacol*. 2023;12(1):134-140. doi:10.18203/2319-2003.ijbcp20223368.
14. Fan J, Zhang K, Jin Y, Li B, Gao S, Zhu J, et al. Pharmacological effects of berberine on mood disorders. *J Cell Mol Med*. 2019;23(1):21-28. doi:10.1111/jcmm.13930.
15. Turner RA. *Screening Methods in Pharmacology*. New York: Academic Press; 1965.
16. Bourin M, Hascoet M. The mouse light/dark box test. *Eur J Pharmacol*. 2003;463(1-3):55-65.
17. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985;14(3):149-167.
18. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.
19. Kokate CK. Practical pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994.
20. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). CPCSEA guidelines for laboratory animal facility. New Delhi: Government of India; 2003.
21. Porsolt RD, Bertin A, Jalfre M. Behavioural despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 1977;229(2):327-36.
22. Kulkarni SK. Handbook of experimental pharmacology. 4th ed. New Delhi: Vallabh Prakashan; 2012.
23. Vogel HG. Drug discovery and evaluation: Pharmacological assays. 2nd ed. Berlin: Springer; 2002.
24. Bourin M, Hascoet M. The mouse light/dark box test. Eur J Pharmacol. 2003;463(1-3):55-65.
25. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985;14(3):149-67.
26. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc*. 2007;2(2):322-8.
27. Anser H, Ikram R, Khatoon H, Naeem S, Khan SS, Nazim U, et al. Comparison of the antidepressant like activity of homeopathic remedies (*Argentum nitricum*, *Staphysagria* and *Ignatia amara*) and their effect on the behavior of rodents. Pak J Pharm Sci. 2020;33(3):937-945. doi:10.36721/PJPS.2020.33.3.REG.937-945.1.
28. Fan J, Zhang K, Jin Y, Li B, Gao S, Zhu J, et al. Pharmacological effects of berberine on mood disorders. J Cell Mol Med. 2019;23(1):21-28. doi:10.1111/jcmm.13930
29. Plaingam W, Sangsuthum S, Angkhasirisap W, Tencomnao T. Kaempferia parviflora rhizome extract and Myristica fragrans volatile oil increase the levels of monoamine neurotransmitters and impact the proteomic profiles in the rat hippocampus: Mechanistic insights into their neuroprotective effects. J Tradit Complement Med. 2017;7(4):538-552. doi:10.1016/j.jtcme.2017.01.002.
30. Hacke ACM, Miyoshi E, Marques JA, Pereira RP. Anxiolytic properties of *Cymbopogon citratus* (DC.) Stapf extract, essential oil and its constituents in zebrafish (*Danio rerio*). J Ethnopharmacol. 2020;260:113036. doi:10.1016/j.jep.2020.113036
31. Korte SM, de Boer SF. A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. Eur J Pharmacol. 2003;463(1-3):163-175. doi:10.1016/S0014-2999(03)01279-2.
32. Thakur AK, Shakya A, Husain GM, Emerald M, Kumar V. Gut-microbiota and mental health: current and future perspectives. J Pharmacol Clin Toxicol. 2014;2(1):1016. doi:10.47739/2333-7079/1016.