

Pharmacological Evaluation of Hydroalcoholic Extract of Herbal Drugs for Its Antidepressant Potential

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ABSTRACT

Purpose: Millions of people worldwide struggle with depression, an illness that has the potential risk of causing suicidal tendencies that can lead to death. The current antidepressants available, need several weeks of treatment to cause the remission of depressive symptoms and are associated with multiple side effects. Therefore, there is a growing interest in the investigation of alternative therapeutic approaches or adjunctive strategies which could be useful for the management of depression. Many studies indicated that *Cocculus hirsutus* & *Persea americana* has neuroprotective properties and potential benefits in managing neurological conditions. However, more research is needed to understand its effects on depression. Hence, we evaluated hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* leaves for its antidepressant potential.

Methods: Antidepressant activity of hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* leaves was evaluated using forced swim test, Tail suspension test (TST) and open field test (OFT). Additionally, brain monoamines estimation (i.e. Dopamine, Norepinephrine, and 5-HT) was carried out to understand the mechanism of action.

Result: Treatment with hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* showed significant decrease in immobility time in FST & TST and increase in locomotion in open field test. Further brain amine estimation exhibited increase in NE and 5-HT.

Conclusion: This study suggests that hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* leaves possess significant antidepressant activity by modulating brain NE and 5-HT level. Therefore, hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* can be used as a valuable plant supplement to treat depressive disorders...

Keywords: *Cocculus hirsutus* & *Persea americana*, antidepressant activity, tail suspension test

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INTRODUCTION

Depression is a mood and emotional disorder. It has the potential to impact various activities of life, including interactions with family and community. It also contributes to challenges in academic and professional life. (Institute of Health Metrics and Evaluation) As per the survey about 4% of the population experience depression, including 5% of adults. Approximately more than 200 million people in the world have one of the MDD. (Woody et.al. 2017) Depression is more common in women than men. According to WHO survey pregnant and postpartum women experienced high percentage of depression disorder. More than 700 000 people die due to suicide every year. Suicide is the fourth leading cause of death in young to old population. (Woody et.al. 2017)

Depression is primary marked as an imbalance between monoamine neurotransmitters. (serotonin, dopamine & Nor-adrenaline) Which play the vital role in managing the stress and mood. This neuronal decline results in an

imbalance between dopamine (DP), serotonin & nor-adrenaline levels in the brain, manifesting as the symptoms of Depression. (Bowden, C et.al.1997) Major effects of depression are on a person's thoughts & behaviour. Clinical symptoms commonly observed include motor related is various psychological and non-motor include is sad & irritable mood, loss of interest in daily activities, changed in appetite & sleep. Biological symptoms include altered thought, loss of appetite and action. Emotional factors include feelings of guilt, ugliness, and loss of motivation and a state of constant depression may suggest prolonged stress or a biochemical imbalance may take place. (Ana B et.al. 2017)

Antidepressant drug such as tricyclic antidepressants SSRI is used to treat depression. Available antidepressants showing huge side effects include Serotonin syndrome, Hyponatremia, Persistent sexual dysfunction, Dizziness, and falls, etc. and thus, need to investigate for a new herbal antidepressant without side effects is more beneficial and

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important. (Bowden, C et.al.1997) herbal medicines are finished, labelled medicinal products that contain active ingredients from aerial or underground parts or other plant materials, or a combination therefore, whether in the crude state or as plant preparations. Demand for the herbal medicine sector is growing fast, increasing by around 15% value per year. (Badawy, Aet.al.1981) A considerable portion of depressed individuals do not stick to their treatment due to a variety of reasons like numerous drawbacks of the antidepressants which are already available in the market, therefore there is growing interest to find out alternative therapy which is becoming more popular or complementary techniques that might be helpful in the treatment of depression. (Bowden, C et.al.1997)

Therefore, this proposal reviews evidence on the antidepressant effects elicited by natural compounds belonging to polyphenol and terpenoid classes and their mechanisms of action. (Badawy, Aet.al.1981) Polyphenols (such as curcumin, rutin, rosmarinic acid, apigenin, epigallocatechin gallate and other green tea polyphenols, hesperidin, resveratrol, and quercetin) have neuroprotective properties, which make them promising complementary compounds for the treatment of psychiatric disorders. (Badawy, Aet.al.1981) Terpenoids (such as hyperforin, ginsenosides, linalool, ursolic acid, and amyryns) constitute another category of phytochemicals that have been shown to produce antidepressant and anxiolytic effects. This proposal also provides the status of clinical studies that, although scarce, present evidence on the possible beneficial effects of some polyphenols and terpenoids for the treatment of depressive and anxiety disorders. (Ana B et.al. 2017)

There is growing interest in the discovery and study of extracts and phytochemical compounds with therapeutic biological properties related to the treatment of psychiatric disorders, including depression. (Bowden, C et.al.1997) This interest is mainly since conventional antidepressants cause several side effects and are not effective in treating a significant percentage of depressive disorder in individuals, besides requiring several weeks of treatment to cause the remission of depressive symptoms. The widespread availability of multiple nutraceuticals generated from polyphenols and terpenoids, which are secondary metabolites produced by plants, is primarily responsible for the positive effects on human health. (Bowden, C et.al.1997, Badawy, Aet.al.1981)

Based on the literature review, epidemiology, and prevalence of the CNS disorder, there is a need to explore more herbal medicinal plants for their antidepressant potential. (Nayak S et.al.2003) Today, extensive research is being conducted worldwide on plant-based medicines based on two components of Traditional Medicine: Flavonoids and Terpenoids. Most natural drugs are derived from plant and animal sources, most of them from plants. An attempt has been made to evaluate the effect of some herbal medicinal plants for antidepressant activity. (Nayak S. et.al 2019)

In recent decades, much attention has been paid to the study of herbal pharmacology, which has shown a variety of

mechanisms of action used in the treatment of depression, including monoamine reuptake, effects on neuroreceptor binding and neurotransmission, modulation of the hypothalamic-pituitary-adrenal (HPA) axis, etc. Many of such type of research study have been completed in the past. (Ana B et.al. 2017, Badawy, A et.al.1981)

The presence of many phytochemicals such as flavonoids, saponins and unsaturated terpenoids and sterols. It has been demonstrated that flavonoids possess antidepressant effects. The naturally occurring flavonoids and their synthetic derivatives have been reported to selectively bind to the central benzodiazepine receptors and to exert antidepressant and other benzodiazepine-like effects in animals. (Badawy, A et.al.1981)

Leaves of *Cocculus hirsutus* belonging to Menispermaceae family commonly referred as a Jaljamini & *Persea americana* belonging to Lauraceae family is commonly referred as Avocado. Both plants proved to be a good source of triterpenoids, polyphenols & flavonoids. They are also possessing antioxidant & antistress activity. (Abbagoni, S et.al. 2021)

Herbal medicines are an effective therapy for the treatment of different ailments and disorders. (Nayak S. et.al 2019) Phytotherapy includes plants with mild action, like mint and chamomile to potent medicinal plants. These medicinal products are used to treat a wide range of medical conditions, from minor injuries, heart, respiratory and central nervous system disorders to serious carcinogenic conditions. (Abbagoni, S et.al. 2021) Menispermaceae plants have also been reported to be active in this way. While *Cocculus* species (*Indicus*, *Hirsutus* and *Penedulus*) have been shown to be significant for their antidiabetic, anti-bacterial, anti-inflammatory, anti-cancer, anti-hypertensive, diuretic, analgesic, and anxiolytic activities. (Maqbool S et.al 2019)

The species of *Cocculus* and *Persea* are used as ingredients in various formulations in the Ayurveda system of medicine as Guduchi Satwam. It heads the list of valuable bitter tonics in Ayurvedic pharmacopoeia and is the bitterest amongst them. (Maqbool S et.al 2019) It is recommended for various ailments like rheumatism, urinary disease, diabetes, dyspepsia, splenic affections, chronic gonorrhoea, diarrhoea, dysentery, and vermifuge. (Dr. Umamaheswari S, 2014) Traditionally, the root and leaves of *Cocculus hirsutus* & *Persea americana* have more medicinal value. The juice of the leaves coagulates in water, and it is used as a cooling agent for eye problems and a soothing agent in eczema, Impetigo, and dyspepsia. Decoction of the root is used in rheumatism. (Logesh R et.al, 2020)

Although widely used in traditional medicinal products and well-studied for their pharmacological properties, the chemical components of *C. hirsutus* & *Persea americana* have not been studied extensively. Previous studies in the 1960s and 1970s had reported the presence of alkaloids by means of preliminary phytochemical tests and isolation and identified several alkaloids such as trilobine, isotrilobine, coccaurine and magnoflorine and other compounds such as inositol, beta-sitosterol, ginnol and monomethyl ether (Olamide et.al. 2022). Few studies performed in or after the

1980s have reported on a few alkaloids from different parts of the plant. Three flavonoids, rutin, liquiritin and quercetin, have also been reported in leaf samples (Rebecca et.al. 2023) hirsudiol, a triterpenoid derivative, has also been reported in ethanol extract from whole plant. Similarly, the aqueous extract of the aerial parts contained both the beta-sitosterol and 28-acetylbotulin. Many studies have reported preliminary phytochemical testing of extracts and the presence of carbohydrates, steroids, alkaloids, glycosides. (Logesh R et.al. 2020)

MATERIALS & METHODS:

Materials

Plant Material & hydroalcoholic Extraction

The cleaned and dried leaves of plants were collected from a local nursery in Kashti, Ahilyanagar, Maharashtra. Then leaves are sun-dried and ground in a mortar & pestle.

Experimental animals:

The experiment was performed on healthy swiss albino mice of both sexes weighing 20–30 g. All animals were acclimatised to the laboratory environment for two weeks before use. The mice were kept in cages at a controlled room temperature of 27 degrees Celsius (2 degrees Fahrenheit) with an equal light-dark cycle for 12 hours, fed a standard pellets diet and water. The experiments mentioned in this study were carried out in accordance with the guidelines of the local Animal Ethics Committee, and approval was obtained from the Ethics Review Committee SCI/IAEC/2024-25/101.

METHODS

Preparation of extract

Powder of *Cocculus hirsutus* & *Persea americana* is extracted using ethanol (70%) in Soxhlet apparatus. The extract was dried in rotary evaporator and percentage yield of extract was calculated is 12% & 09% for *Cocculus hirsutus* & *Persea Americana* respectively.

Qualitative and quantitative phytochemical analysis

The phytochemical composition of *Cocculus hirsutus* & *Persea Americana* was examined through both qualitative and quantitative methodologies. Initially, the plant material was dried and converted into fine powder, which was then extracted using hydroalcoholic solution. This concentrated extract is used for preliminary phytochemical screening. Qualitative tests confirmed the presence of alkaloids, flavonoids, phenols, and tannins, saponins, and terpenoids. For quantitative analysis, spectrophotometric methods were employed. The total phenolic content was determined to be 24.039mg GAE/g & 51.859mg GAE/g for *Cocculus hirsutus* & *Persea Americana* respectively, using the Folin-Ciocalteu method, while the total flavonoid content was 240.01 mg Quercetin/g. & 209.471mg Quercetin/g for *Cocculus hirsutus* & *Persea Americana* respectively.

Behavioral assessment

Oral toxicity test of hydro alcoholic extract of *Cocculus hirsutus* & *Persea americana* leaves has already been conducted in various research. They were recorded; blood and biochemical parameters were analysed and histopathological studies were concluded that the lethal

effects of the extract after repeated administration to rodents over a 28-day period were assessed by assessment of body weight, blood and biochemical parameters, histological and pathological changes. No significant difference in biochemical parameters was observed between the 100 mg and 200 mg per kg dose levels of extract compared to the control group ($p < 0.05$). (Uma Sankar Gorla et.al. 2021, Prince et.al. 2025)

Drug Administration

The animals were randomized into control and experimental groups and divided into four groups of 06 animals each. Animals in group 1 were administered with normal saline (0.9% NaCl). Animals in group 2 were administered with fluoxetine standard drug (20mg/kg), and animals in groups 3 and 4 were administered with the extracts of *Cocculus hirsutus* & *Persea americana* (100mg/kg, 200mg/kg). All drugs were orally administered. The behavioural tests were conducted 1 h after the last treatment.

Forced swim test: Animals were randomly divided into 6 groups of six animals in each. Group one received normal saline solution for normal control. Group II treated with standard drug fluoxetine (20mg/kg). Group III & IV received *Cocculus hirsutus* extract (100mg/kg & 200mg/kg respectively). Group V & VI received *Persea americana* extract (100mg/kg & 200mg/kg respectively). Immobility time recorded after 1 hr of drug treatment using forced swim test & Tail suspension method. In forced swim test animals were separately forced to swim in open container containing water. (diameter 10 cm, height 25 cm), at 25 ± 1 °C; and the immobility time (total time in 6 minutes was recorded as when the mice became immobile) was recorded. (S. K. Kulkarni, R D Porsolt et.al. 1977, Kim J. W et.al. (2024)

Tail suspension test: Same treatment was given to animals as described in forced swim test. Immobility time was measured the tip of the tail of the animal was be taped and suspending it 30 cm from the floor, and the duration of immobility behaviour of the animals was observed for 5 minutes. (S. K. Kulkarni, R D Porsolt et.al. 1977)

Open-field test in: After the tail suspension method, the same animal was treated with drugs for 28 days and then evaluated in the open-field test for the exploratory behaviour by estimating the number of squares crossed (crossings) by mice were counted and recorded in a 6-minute session. (R D Porsolt et.al. 1977, Z.F. Yu et.al. 2002, Sakakibara H. 2006)

Biochemical estimation: After completion of open field behavioural tests, all animals were sacrificed; the brain samples were carefully isolated, and immediately kept in cold buffer solution (pH 7.0). The brain samples were homogenized and centrifuged (Neaution, India) at 10000 RPM at 40-45°C for 10 minutes. Supernatants liquid was separated and used for the estimation of serotonin, dopamine, and noradrenaline. Serotonin, dopamine, and nor-adrenaline were estimated by the procedure described on the leaflet of the respective ELISA kits. The results were expressed as pg/mg of tissue.

Evaluation of brain bioamine estimation:

Haloperidol induced catalepsy (dopamine-mediated behaviour): Mice were randomly divided into groups of 6 (six in each). The control group treated with NS (1 ml/kg orally) and haloperidol (30mg/kg i.p.). The std groups treated with standard drug Dopamine before administration of haloperidol. Group III & IV received CH extract of 100mg/kg & 200mg/kg, respectively and Group V & VI received PA extract of 100mg/kg & 200mg/kg, respectively, 30 mins before haloperidol induced. Record the time for correcting the posture of mice in the intervals of 0, 30, 60, 90, 120 mins. (Iyer M.R et.al. 1998, Bhatwadekar A.D et.al 1999)

Clonidine induced hypothermia (Nor-adrenaline-mediated behaviour): Mice were randomly divided into groups of 6 (six in each). Rectal temperature was recorded every 30 minutes after clonidine (0.1mg/kg ip) administration till 120 minutes. Imipramine(20mg/kg) was used as a standard drug. Groups III & IV received CH extract of 100mg/kg & 200mg/kg, respectively and Groups V & VI received PA extract of 100mg/kg & 200mg/kg, respectively, were administered 30 minutes before clonidine. (Iyer M.R et.al. 1998, Bhatwadekar A.D et.al 1999)

Lithium-induced head twitches (Serotonin-mediated behavior): Mice were treated with lithium sulphate (200mg/kg i.p.) 30 minutes after treatment with CH & PA (100 & 200 mg/kg po). The head twitches count was recorded for 60 minutes after the lithium sulphate treatment. (Iyer M.R et.al. 1998, Bhatwadekar A.D et.al 1999)

Statistical Analysis:

Results were expressed as Mean \pm SEM (n=6). Data was analysed by one-way ANOVA followed by Bonferroni post hoc analysis. $p < 0.05$ was considered statistically significant.

Results:

Effects of the hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* on the duration of immobility time in the forced swim test: - After the oral administration of the hydroalcoholic extract of *Cocculus hirsutus*, *Persea americana*, and standard drug fluoxetine shows effect on the duration of immobility in the mice in forced swimming test are shown in Table no 01.

As per the experimental study the hydroalcoholic extract at the doses of 100 mg/kg and 200mg/kg exhibited to show significant reduction in immobility time. The extracts at doses of 100 mg/kg showed decreased the time of immobility as compared to 200mg/kg. The effect of *Cocculus hirsutus* and *Persea americana*, at the dose of 100 mg/kg, appeared to be more potent than that of fluoxetine.

Effects of the hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* on the immobility time in

the Tail suspension test: - treatment of oral administration of the hydroalcoholic extract of *Cocculus hirsutus*, *Persea americana*, and fluoxetine on the time of immobility in the mice using tail suspension test are shown in Table no 02. The extract at the doses of 100 mg/kg and 200mg/kg exhibited to show reduction in immobility time. The extracts at doses of 100 mg/kg significantly decreased the duration of immobility. The effect of *Cocculus hirsutus* and *Persea americana* at the dose of 100 mg/kg appeared to be more potent than that of fluoxetine.

Effects of the hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* on the duration of immobility time in the Open Field Test: - After the 28 days of treatment of drugs on animal groups the Effects of the hydroalcoholic extract of *Cocculus hirsutus*, *Persea americana*, and fluoxetine on the behavioural study in the mice in open field test were shown in Table no 03. Significant difference in ambulation, rearing, and the number of grooming in the open-field behaviour test, compared with the normal control group. The extract at the doses of 100 mg/kg and 200mg/kg exhibited to show an increase no. of crossings compared to the normal control group. But no significant changes compared to the standard control group.

Effects of the hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* on brain bioamine estimation: - Our experimental findings demonstrated that, brain monoamines, mainly serotonin, dopamine and norepinephrine levels were found to be increased levels of the monoaminergic neuron as compared to the normal control group, as shown in Table no 4.

Evaluation of brain bioamine estimation

Haloperidol induced catalepsy (dopamine-mediated behaviour): - In vehicle-treated mice, haloperidol induced maximum catalepsy after 60 min. Prior administration of CH & PA (100mg/kg & 200mg/kg) decreased the intensity of catalepsy without affecting the time required to show maximum catalepsy. With the lower dose of CH & PA i.e. 100mg/kg, the intensity of catalepsy was reduced.

Clonidine induced hypothermia (Nor-adrenaline-mediated behaviour): -hypothermia: In vehicle treated mice, clonidine produced a fall in rectal temperature, CH & PA modify the hypothermia in mice produced by the clonidine.

Lithium-induced head twitches (Serotonin-mediated behavior): In vehicle treated rats, lithium sulphate produced 26.0 \pm 2.61 head twitches. The administration of CH and PA decreased the number of head twitches to 1.75 \pm 0.62 and 11.5 \pm 1.04 respectively ($P < 0.05$).

Table 01 & 02: - Effects of *Cocculus hirsutus* and *Persea americana* and fluoxetine on immobility time in the forced swim and tail suspension test in mice.

Treatment and Dose	Immobility time (Sec; Mean \pm SD)	
	FST	TST
Normal Control	189.83 \pm 36.20	198.16 \pm 11.65

Standard –Fluoxetine 20 mg/kg	132.83 ±10.64**	168.16 ± 07.85***
<i>Cocculus hirsutus</i> extract, 100 mg/kg	139.16 ± 30.34**	173.66 ± 09.43**
<i>Cocculus hirsutus</i> extract, 200 mg/kg	143.66 ±14.50*	168.5 ± 06.44***
<i>Persea americana</i> extract, 100 mg/kg	119.5 ± 28.45***	126.66 ± 15.80****
<i>Persea americana</i> extract, 200 mg/kg	143.16 ± 11.75*	174.66 ± 07.17**
Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ****P<0.0001, ***P<0.0001, **P<0.001, compared to vehicle control.		

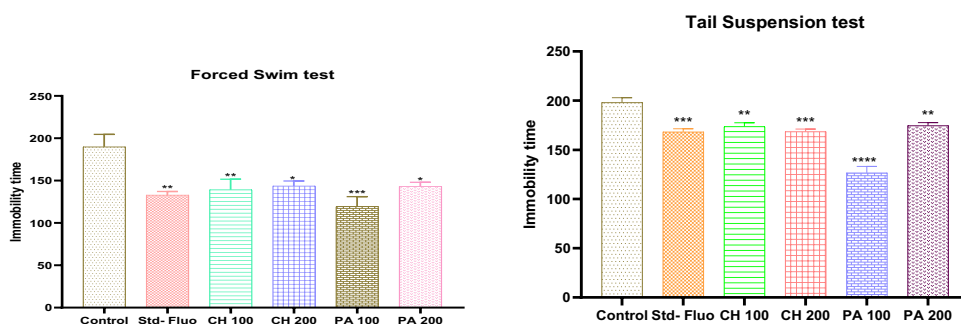


Fig no: -01&02. Effects of *Cocculus hirsutus* or *Persea americana* or fluoxetine on immobility time in forced swim and tail suspension test in mice. Data is expressed as Mean±SD (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, *** P<0.001, ** P<0.01, * P<0.1 compared to normal control

Table 03: - Effects of *Cocculus hirsutus* or *Persea americana* or fluoxetine Number of crossings in Open Field Test in mice.

Treatment and Dose	Number of crossings (number) (Sec; Mean±SD)
Normal Control	137.33 ± 22.60
Standard –Fluoxetine 20 mg/kg	168.5 ± 6.65
<i>Cocculus hirsutus</i> extract, 100 mg/kg	162.8333 ± 6.21
<i>Cocculus hirsutus</i> extract, 200 mg/kg	148.8333 ± 6.58
<i>Persea americana</i> extract, 100 mg/kg	157.5 ± 6.53
<i>Persea americana</i> extract, 200 mg/kg	148.3333 ± 12.35

Effects of *Cocculus hirsutus*, *Persea americana* and fluoxetine on no of crossing in Open-field test in mice. Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, * P<0.05 compared to normal control

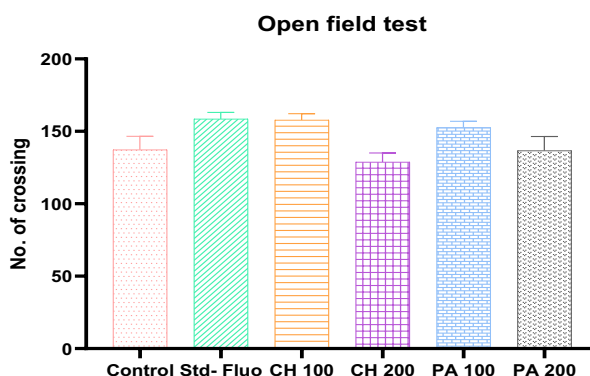


Fig. no: -03 Effects of *Cocculus hirsutus*, *Persea americana* or fluoxetine on no of crossing in Open-field test in mice. Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, * P<0.05 compared to normal control

Table 04: - Effects of *Cocculus hirsutus*, *Persea americana*, or fluoxetine biochemical estimation in mice.

Treatment and Dose	Brain monoamines (µmol/L; Mean±SD)		
	Serotonin	Dopamine	Norepinephrine
Normal Control	162.33±19.82	156.66±37.46	148.66±38.52
Standard –Fluoxetine 20 mg/kg p/o	226.83±29.73**	236.33±18.47*	226.00±20.21**
<i>Cocculus hirsutus</i> extract, 100 mg/kg p/o	225.16±18.99**	232.16±35.93*	211.5±25.54*
<i>Cocculus hirsutus</i> extract, 200 mg/kg p/o	231.00±29.11***	227.66±27.35*	215.83±25.54**
<i>Persea americana</i> extract, 100 mg/kg p/o	229.33±35.07**	237.33±77.95*	241.16±45.27***
<i>Persea americana</i> extract, 200 mg/kg p/o	232.83±28.93***	224.00±27.29*	221.00±47.57**

Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ***P<0.0001, **P<0.001, *P<0.01 compared to normal control.

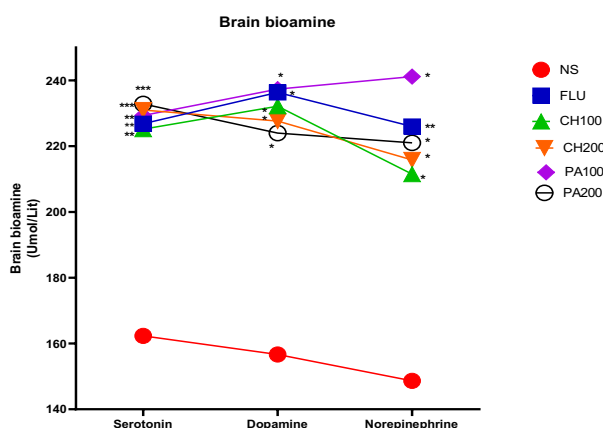


Fig. no: -04 Effects of *Cocculus hirsutus*, *Persea americana* or fluoxetine on brain bioamine estimation in mice. Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, *P<0.0001, **P<0.001, *P<0.01 compared to normal control.**

Table 05: - Effects of *Cocculus hirsutus*, *Persea americana*, or fluoxetine on haloperidol induced catalepsy in mice.

Treatment and Dose	Time to correct posture (sec)				
	00 min	30 min	60 min	90 min	120 min
Vehicle + Haloperidol 20 mg/kg	82.5 ± 2.66	112.66 ± 14.94	113.33 ± 6.94	102.16 ± 8.52	112.83 ± 6.94
Levodopa (30 mg/kg) + Haloperidol	80.66 ± 2.16	76.66 ± 5.35****	98.33 ± 2.94***	82.5 ± 5.16***	95 ± 5.58****
<i>Cocculus hirsutus</i> extract, 100 mg/kg + Haloperidol	81.5 ± 1.87	91.33 ± 6.91***	102 ± 5.29**	88 ± 5.54**	98.33 ± 7.14**
<i>Cocculus hirsutus</i> extract, 200 mg/kg + Haloperidol	81.33 ± 2.94	95.83 ± 8.20**	100.33 ± 6.65**	87.33 ± 5.85**	98 ± 3.57***
<i>Persea americana</i> extract, 100 mg/kg + Haloperidol	80.66 ± 1.75	89.5 ± 6.28***	99.5 ± 2.73**	88.16 ± 5.91**	98 ± 4***

Persea americana extract, 200 mg/kg + Haloperidol	80.83 ± 2.56	94.5 ± 5.35**	99.16667 ± 2.401388**	89.5 ± 11.46*	99.33 ± 7.65**
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Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ***P<0.0001, **P<0.001, *P<0.01 compared to normal control.

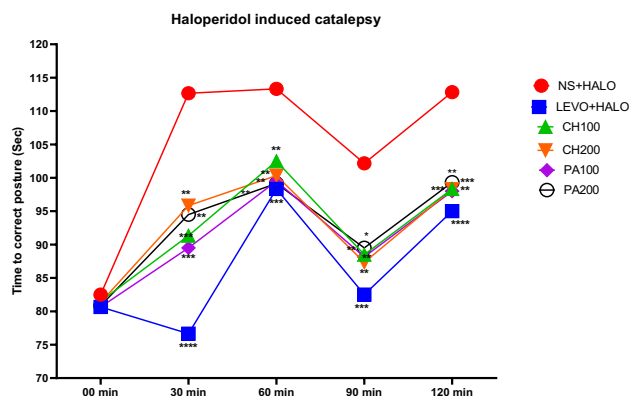


Fig. no: -05 Effects of *Cocculus hirsutus*, *Persea americana* on Haloperidol induced catalepsy in mice, Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ***P<0.0001, **P<0.001, *P<0.01 compared to normal control.

Table 06: - Effects of *Cocculus hirsutus*, *Persea americana*, on Clonidine Induced Hypothermia in mice.

Treatment and Dose	Temp °C			
	30 min	60 min	90 min	120 min
Vehicle + Clonidine 0.1 mg/kg	29.3 ± 0.77	29.2 ± 0.33	28.73 ± 0.60	28.32 ± 0.53
Clonidine + Imipramine 20mg/kg	30.75 ± 0.27***	30.52 ± 0.54****	30.48 ± 0.48****	29.87 ± 0.34****
<i>Cocculus hirsutus</i> extract, 100 mg/kg	30.37 ± 0.62**	30.18 ± 0.55**	29.83 ± 0.34**	29.48 ± 0.49**
<i>Cocculus hirsutus</i> extract, 200 mg/kg	30.31± 0.31**	30.10 ± 0.17**	30.05 ± 0.25****	29.55 ± 0.40***
<i>Persea americana</i> extract, 100 mg/kg	30.28 ± 0.54*	30.2 ± 0.47**	29.86 ± 0.48***	29.4 ± 0.38**
<i>Persea americana</i> extract, 200 mg/kg	30.37 ± 0.37**	30.38 ± 0.50***	29.76 ± 0.41**	29.45 ± 0.62**

Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ***P<0.0001, **P<0.001, *P<0.01 compared to normal control.

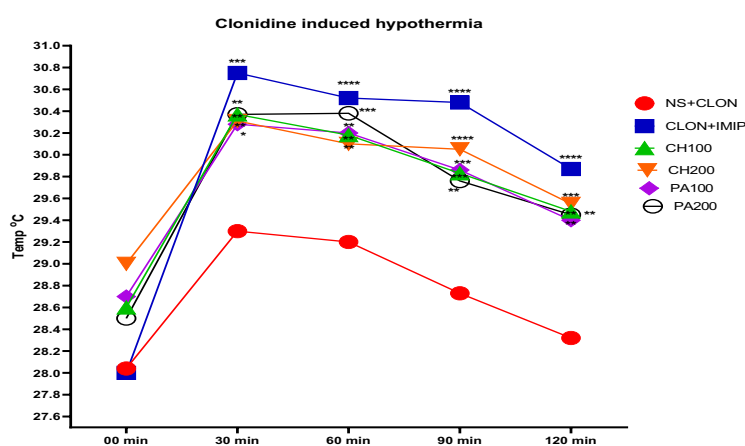


Fig. no: -06 Effects of *Cocculus hirsutus*, *Persea americana* on Clonidine induced hypothermia, Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ***P<0.00001, **P<0.001, *P<0.01 compared to normal control.

Table 07: - Effects of *Cocculus hirsutus*, *Persea americana*, on Lithium induced head twitches in mice.

Treatment and Dose	Number of head twitches in one hour
Vehicle + Lithium sulphate 200 mg/kg	30.33 ± 2.06
Ketanserine 10mg/kg + Lithium sulphate 200 mg/kg	24.33 ± 1.75****
<i>Cocculus hirsutus</i> extract 100 mg/kg + Lithium sulphate 200 mg/kg	27.16 ± 1.169*
<i>Cocculus hirsutus</i> extract 100 mg/kg + Lithium sulphate 200 mg/kg	26.16 ± 2.22**
<i>Persea americana</i> extract 100 mg/kg + Lithium sulphate 200 mg/kg	26.16 ± 1.94**
<i>Persea americana</i> extract 200 mg/kg + Lithium sulphate 200 mg/kg	26.16 ± 1.47**

Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, ****P<0.00001, **P<0.001, *P<0.01 compared to normal control.

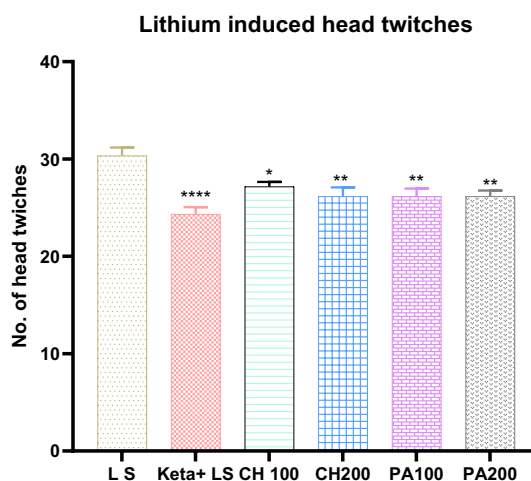


Fig. no: -07 Effects of *Cocculus hirsutus*, *Persea americana* on lithium induced head twitches, Data is expressed as Mean±SEM (n=6), analyzed by One Way ANOVA followed by Bonferroni Post Hoc analysis, **P<0.00001, **P<0.001, *P<0.01 compared to normal control.**

DISCUSSION:

This study showed the antidepressant effects of *Cocculus hirsutus* & *Persea americana* through behavioural animal models, addressing the significant challenge of developing a valid model for accurately identifying various antidepressant treatments. In this study, three animal models are used like forced swimming test (FST) developed by Porsolt et al., tail suspension test (TST) in mice developed by Steru et al., and open field test by Ani Kristiyani et.al.

The present study findings demonstrate for the first time that a hydroalcoholic extract of a *Cocculus hirsutus* & *Persea americana* demonstrates significant antidepressant-like effects in mice when administered systemically in the FST, TST, and OFT of depression models. The antidepressant-like effect produced by the extract of *Cocculus hirsutus* & *Persea americana* shows most likely to be mediated through an interaction with adrenergic, dopaminergic, and serotonergic systems. This evidence came from several in vitro and in vivo data. As per our study investigated the involvement of the serotonergic, noradrenergic, and dopaminergic systems showed its

actions of immobility effect in the FST, & TST and number of square crossings in OFT. The hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* produced a significant antidepressant-like response in FST, OFT, and TST. After that the repeated administration (28 days) of the hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* was also able to produce an antidepressant-like effect in the OFT in mice. The extract of this plant had an effect like that of the standard antidepressant fluoxetine (20 mg/kg, p.o.). our results shows that the reduced immobility time in mice in forced swim test and tail suspension model is 100gm/kg of hydroalcoholic extract of *Cocculus hirsutus* & *Persea americana* in forced swim test and tail suspension model, and 200mg/kg of hydroalcoholic extract *Cocculus hirsutus* & *Persea americana* in Open field test. Boosting brain monoamine neurotransmitters is widely recognized as an effective treatment for depression, which is often linked to the dysfunction of noradrenaline, serotonin, and dopamine in the CNS, referring to the monoamine hypothesis. Generally, the most widely accepted hypotheses of the biological basis of depression implicate the 5-HT and noradrenaline system dysfunction. In our study, we detected the brain monoamine level by bioamine estimation after 21 days of treatment. The serotonergic system is intimately

linked to stress and depression responses. (Drevets, W.C. et al.2001) Besides the well-established role of the serotonergic system in the pathogenesis of depression, this system is also a target for conventional pharmacotherapy. (Graeff, F et.al.1996) Lithium induced head-twitch tests, Clonidine Induced Hypothermia, and Haloperidol-induced catalepsy are usually used to investigate the effects of antidepressant drugs on serotonergic, noradrenergic, and dopaminergic function. (An, L et.al.2008) This test provides a simple method of determining the central action of 5-HT, N-Ad, and Dopamine. The present study revealed that *Cocculus hirsutus* & *Persea americana* extract treatment significantly increased the number of head-twitches of mice induced by 5-HTP, reduced haloperidol-induced catalepsy, and increased body temperature in clonidine-induced hypothermia. Therefore, we assumed that the serotonergic, Dopaminergic, and Noradrenergic systems are involved in the antidepressant-like effects of *Cocculus hirsutus* & *Persea americana* extract.

CONCLUSION:

In the present experimental research work, we demonstrated the potential effects of *Cocculus hirsutus*, *Persea americana*, and fluoxetine as antidepressants in mice using various animal models. Antidepressant activity was evaluated by estimating the immobility time. Our experimental findings demonstrated that immobility time in forced swim and tail suspension tests was found to increase significantly ($P < 0.001$) compared to the normal control group (Table 1&2; Figure 1&2). Pretreatment with *Cocculus hirsutus*, *Persea americana*, and fluoxetine exhibited attenuation of immobility time in dose dependent manner compared to the vehicle control group.

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Further, number crossing was found to exhibit non-significant changes in the open field test (Table 3, Figure 3), suggesting the antidepressant activity of *Cocculus hirsutus*, *Persea americana*, and fluoxetine, independent of crossing behaviour. Furthermore, to corroborate the antidepressant like activity of *Cocculus hirsutus*, *Persea americana*, and fluoxetine by estimating the brain monoamine levels. Our experimental findings demonstrated that brain monoamines, mainly serotonin, dopamine, and norepinephrine levels, were found to be increasing monoamines in the treatment groups. Thus, in conclusion, our findings revealed that *Cocculus hirsutus* & *Persea americana* exhibited antidepressant like activities in various animal models in the present study, which are independent of brain monoamine levels.

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Compliance with ethical standards:

Conflict of interest:

The authors declare that there is no conflict of interest.

Statement of animal rights

The protocol got sanctioned by the Institutional Animal Ethics Committee at Scitesla Pvt Ltd, TTC, MIDC, Navi Mumbai. (Reg no: SCI/IAEC/2024-25/101.)

Credit authorship contribution statement

Pallavi Jadhav: Conceptualization, Writing– original draft, Methodology, Software, Suvarna Ingale: Supervision, Writing review & editing, Formal analysis

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