Evaluation of Anti-depressant Activity of *Actaea Racemosa* root in Reserpine-induced experimental animal model

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ABSTRACT

Background: *Mental health has always been a Prime importance along with physical health by the World Health Organization (WHO). Depression contributes to Significant disease burden at global level and is ranked by WHO as the single largest contributor to global disability. Actaea racemosa is known for menopausal properties, hence, this study has been conducted to evaluate the antidepressant activity of Actaea racemosa root extract in rodent models.*

Materials and Methods: *Actaea racemosa root extract was used for evaluating the Antidepressant activity in Swiss Albino Mice. Antidepressant activity was evaluated by comparing with the positive control group by performing Forced swim test and Tail suspension test with Imipramine (10mg/kg) as the standard drug. Reserpine is used as an inducing agent. The Data was analyzed using one way ANOVA (analysis of variance) Followed by Dunnett's Multiple Comparison Test. P value <0.0001 was considered as statistically significant.*

Results: *Forced swim test and Tail suspension test showed Significant decrease in the period of immobility by Actaea racemosa treated groups indicating its antidepressant property. There is no significant difference observed between the two doses of the extract i.e.; 20mg/kg and 30mg/kg.*

Conclusion: *This study shows that extract of Actaea racemosa root extract possess significant antidepressant properties. The effect produced by the two doses of the test drug was compared with the standard drugs Imipramine and along with the plain control adopting appropriate statistical analysis.*

Keywords: *Antidepressant, Actaea racemose, Swiss albino mice.*

1. Introduction

Mental disorders have caused a major disease burden to the world and have increased in the past few years. World Health Organization (WHO) defined psychological health as, "a state of wellbeing in which an individual realizes their own abilities, can withstand the normal stresses of life, can work fruitfully and productively, and can contribute to his or her community.[1] Depression is a psychiatric disorder characterized by tiredness, feelings of guilt, low self-esteem, and sadness, loss of interest, disturbed sleep, disturbed appetite, and poor concentration. Nearly 15 million individuals in India alone experience major mental health issues, while 30 million experience mild to moderate issues (WHO, 2001). Only a small proportion of the 450 million individuals who suffer from mental or behavioural disorders, according to the World Health Report (WHO, 2001), receive even the most basic care. By 2020, this is anticipated to increase to 15% of the global burden of disease. It currently accounts for 12.3% of that burden.[2]

Actaea racemosa is a well-known medicinal Plant since ancient times for its Medicinal properties. Actaea racemosa is native to eastern and central North America, and corresponds to the family Ranunculaceae. It is also distributed in Canada and China, and cultivated in Europe. It is often called black cohosh, bugbane, rattle weed, snakeroot, squaw root, or rheumatism weed. [3] Roots and Rhizome of this plant have been used for the treatment of menopausal symptoms, including hot flashes and night sweats (together known as vasomotor symptoms), vaginal dryness, heart palpitations, tinnitus, vertigo, sleep disturbances, nervousness, and irritability, to treat musculoskeletal pain, fever, cough, pneumonia, sluggish labour, and menstrual irregularities, reduce

musculoskeletal pain and spasms, support liver function, support nervous systems. Sore throats, depression.[4][5] The roots of Actaea racemose are of a great Medicinal value.

Actaea racemosa is known for menopausal properties, and on literature search, there were no studies on antidepressant activity of the plant. Hence, the current study has been conducted to evaluate the anti-depressant activity of Actaea racemose root extract in rodent models of depression for validating its effectiveness scientifically.

2. Materials and Methods

2.1 Animals

Experiments were performed in accordance with the committee for the purpose of control and supervision of experimental animals (CPCSEA) guidelines after the approval of the experimental protocol by the Institutional Animals Ethical Committee (IAEC). The Swiss albino mice (weighing 25-30g), Which are obtained from the animal house of Department of Pharmacology, Vidyabharti College of Pharmacy, Amravati. All the animals are acclimatized to the animal prior to use.

2.2 Drugs and Chemicals

Imipramine, Reserpine were obtained from M.G Pharma Amravati. All the treatment (test drug, standard drug and the vehicle) were given either by oral route or ip with the help of gastric canula or by ip injection. Drugs and vehicle were given in the form of liquid suspension which was freshly prepared at the time of administration to the animals.

2.3 Plant material

The crude drug extract of plant were purchased from Natural Hub Pvt Ltd, New Delhi.

2.4 Phytochemical analysis

The extract obtained from the powdered of *Actaea racemosa* root was subjected to phytochemical tests to determine the presence of active metabolites using standard procedures.[6][7]

2.5 Acute oral toxicity study

The acute toxicity of prepared extract was already performed using **OECD 425 guideline** in a following manner.

2.6 Study Design

For carrying out antidepressant activity by performing forced swimming test (FST) and tail suspension test (TST), a total of 30 Swiss albino mice were randomly divided into 5 groups with 6 animals in each group (either sex). Different sets of animals were used for each experiment. The total number of animals used in the experiment were 30.

1. **Group I**: - Negative Control (Received vehicle, po)
2. **Group II:** - Positive Control (Reserpine 0.5mg/kg, ip)
3. **Group III:** - Standard (Imipramine 10mg/kg, po)
4. **Group IV:** - Actaea racemose low dose (20 mg/kg, po)
5. **Group V:-** Actaea racemose High dose (30 mg/kg, po)

2.7 Experimental procedure

Two doses were taken for Evaluating the antidepressant activity i.e., 20 mg/kg and 30 mg/kg. The quantity of the test drug/extract administered was calculated according to the bodyweight of the animals and was administered orally for a period of 14days. On the 14th day, after one hour of drug administration, animals were tested for antidepressant activity by performing forced swimming test (FST). After that, a washout period of 6 weeks has been given. After the washout period, again, test drug/extract was administered to the animals for a

period of 14 days. On the 14th day, after one hour of drug administration, animals were tested for antidepressant activity by performing tail suspension test (TST).

2.8 Forced Swimming Test

Mice were housed in individual polypropylene cages one day before the experiment. Each mouse was brought into a vertical plexiglass cylinder (40 cm x 18cm and containing 15 cm of water at 25°C) and was observed for 6 minutes. The evaluation was initiated after 2 minutes. Initially, mice were hyperactive, vigorously swimming in circles, trying to climb the wall, or diving to the bottom. After 2-3 min this activity subsided, and there was a period of immobility or floatation of increased duration. Duration of immobility was noted for each mouse. The antidepressant activity was represented by an increase in the duration of mobility or a decrease in the duration of immobility. After the test, animals were allowed to dry and returned to their home cages.

2.9 Tail Suspension Test

Each mouse was suspended upside down 50 cm above the ground with the help of a stand and using an adhesive tape placed approximately 1 cm from the tip of the tail. They were observed for 5 minutes. Mice were considered immobile when it was hanging freely without making any movements. Duration of immobility was noted for each Mouse. The antidepressant activity was represented by an increase in the duration of mobility or a decrease in the duration of immobility. After the test, the animals were returned to their home cages.[8]

2.10 Statistical Analysis

Observations made in FST and TST were compiled and tabulated using the statistical software, GraphPad. Results were represented as Mean ± SEM (Standard Error of Mean). One way analysis of Variance (ANOVA), multiple comparison between the groups were made with Dunnett's Multiple Comparison Test. P value <0.0001 was considered as statistically
significant.

3. Results

3.1 Phytochemical Analysis

On preliminary phytochemical analysis, Actaea racemosa showed the presence of alkaloids, flavonoids, Glycosides, Phenolic compounds, Triterpenoids, Tannins, etc

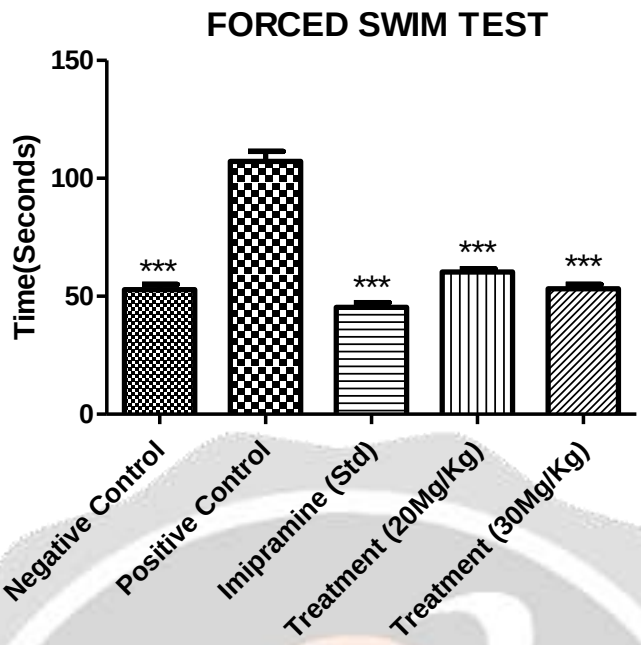
3.2 Acute oral toxicity study

The acute toxicity of prepared extract was already performed using **OECD 425 guideline** in a following manner.

3.3 Forced Swimming Test

Analysis of behaviour of mice in the Forced swimming test revealed that treatment of Imipramine (10 ml/kg), Actaea racemosa (20 and 30 mg/kg) produced significant (***p<0.0001) decrease in duration of immobility as compared to vehicle.

Group	Dose (mg/kg)	Forced Swimming Test (Duration of immobility)
Negative control	Received vehicle (Saline water 10ml/kg)	52.83±2.166
Positive control	Reserpine (0.5 mg/kg)	107.16±4.214
Standard drug	Imipramine(10mg/kg)	45.33±1.943
Treatment group I	Actaea racemosa 20mg/kg	60.33±1.333
Treatment group II	Actaea racemosa 30mg/kg	53.16±1.833



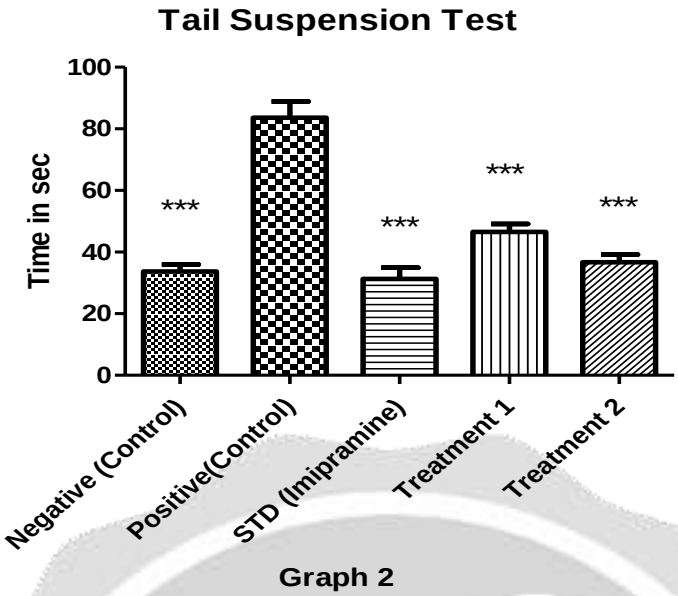
Graph 1

All values are expressed as mean ± S.E.M. of N=5. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test, (***p<0.0001) when compared with vehicle.

3.4 Tail Suspension Test

Analysis of behaviour of mice in the Tail suspension test revealed that treatment of Imipramine (10 ml/kg), Actaea Racemosa (20 and 30 mg/kg) produced significant (****p<0.0001) decrease in duration of immobility as compared to vehicle.

Group	**Dose (mg/kg)**	**Tail suspension Test (Duration of immobility)**
Negative control	Received vehicle (Saline water 10ml/kg)	33.66± 2.66
Positive control	Reserpine (0.5 mg/kg)	83.67±6.113
Standard drug	Imipramine(10mg/kg)	31.33±4.240
Treatment group I	Actaea racemosa 20mg/kg	46.5±3.127
Treatment group II	Actaea racemosa 30mg/kg	36.67±2.973



Graph 2

All values are expressed as mean ± S.E.M. of N=5. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test, (****$p<0.0001$) when compared with vehicle.

4. Discussion

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and physical well-being. Depressed people may feel sad, anxious, empty, hopeless, helpless, worthless, guilty, irritable, or restless. They may lose interest in activities that once were pleasurable, experience loss of appetite or overeating, or problems concentrating, remembering details or making decisions and may contemplate or attempt suicide. According to World Health Report (WHO, 2001) approximately 450 million people suffer from a mental or behavioural disorder, yet only a small minority of them receives even the most basic treatment. This accounts for 12.3% of the global burden of disease and is expected to rise to 15% by the year 2020.
Therefore, *Actaea racemosa* has been studied comprehensively for its antidepressant activity using various tests like Forced Swim Test, Tail suspension test, in mice with different pathophysiological aspects and the mechanisms associated with depression.
These tests have proved the significant antidepressant activities due to the presence of phytochemical like flavonoids, alkaloids, triterpenoids, glycosides, phenolic constituents in *Actaea racemosa.*

In the present study the test drug *Actaea racemosa* was undertaken to illustrate its effect on monoamine reuptake. As the monoamine hypothesis and the neuro imaging techniques have revealed that in depression, there is a deficiency of neurotransmitter serotonin in the brain, which can be altered by antidepressants.

In TST and FST, Actaea racemosa (20 and 30 mg/kg, po) decreased the immobility periods significantly ($p<0.0001$) in a dose dependent manner, when compared to vehicle treated group, indicating significant antidepressant activity.

5. Conclusion

From this study, it can be concluded that Evaluation of Anti-depressant Activity of *Actaea Racemosa* root in Reserpine-induced experimental mice involving specific parameters to determine the activity of plant. To explore the potential of *Actaea racemosa* as an antidepressant, the efficacy of the two doses (20mg/kg & 30mg/kg) of test drug was investigated in Swiss Albino mice through different tests. Its effect on antioxidant, Fertility improvement, Menopausal hot flushes, Sleep disorders etc was evaluated in the previous study to determine the nature of the pharmacological effect produced by the test drug. The present study was focused on specific tests to validate the antidepressant activity of *Actaea racemosa.* The effect produced by the two doses of the test drug was compared with the standard drugs Imipramine and along with the plain control adopting appropriate statistical analysis.

Test drug produced significant degree of antidepressant effect and thus validated the Ayurveda claim that *Actaea racemosa* is an important drug to be useful in the management of depressive disorder.

Actaea racemosa Significantly reduced immobility period in the forced swim and tail suspension tests in mice, which suggests antidepressant effect. Immobility is believed to reflect either a failure to persist in escape directed behaviour after persistent stress or the development of passive behaviour that disengages the animal from active forms of coping with stressful events. The FST and TST are valid behavioural models used frequently to evaluate the potential efficacy of prospective antidepressant drugs in rats or mice. Serotonin have been identified as the neurochemical substrates involved in the mediation of behavioural response in the FST and TST. The behavioural immobility is selectively decreased by a *Actaea racemosa* possess antidepressant activity.

Antidepressant activity of *Actaea racemosa* has been validated based on mechanism of action on comparison with the standard drugs Imipramine thus *Actaea racemosa* is a safe and effective antidepressant through Ayurveda system of Medicine.

6. References

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