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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ETHANOLIC EXTRACT OF PULSATILLA NIGRICANS IN RATS

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Article Info

Abstract

To analyze the traditional and scientific Last significance of Pulsatilla nigricans Linn, a tiny medicinal herb that could be used to treat many ailments either male equally It is respected in traditional Chinese medicine and its a heightened ability to relieve psychological disorders such as anxiety, depression, as well as mental health problems. These traditional ideas have stimulated modern scientific research to prove the herb's potential therapeutic properties. This study is aimed at investigating whether an ethanol extract of Pulsatilla nigricans leaves that has been extracted by the 70% method is effective in treating depression. Phytochemical studies often make use of extraction with ethylene (ethylene gallate), which is an efficient means of isolating the majority of active plant components. Those who were tested on the extract were given two doses, 250 mg/kg and 500 mg/kg. The species involved is likely a rodent, but its identity remains unknown. Two behavioral models, the Tail Suspension Test (TST) and the Forced Swim Test (FST), were employed to evaluate antidepressant activity. These models also measure time of immobility and show lower levels, which corresponds to activity corresponding to antidepressants. Treatment outcomes were significantly better for the treated group than for the control group, which supports the antidepressant properties of the extract. The results of this research offer evidence for the traditional use of Pulsatilla nigricans for mental health and highlight its potential as a natural antidepressant substitute. Additionally, further investigations could aid in identifying the active compounds that caused these effects and verifying the extract's safety and effectiveness on humans.

Keywords:

Pulsatilla nigricans, Anti-depressant, Medicinal Herbs, behavioral skills TST and FST.

Introduction

Depression is the fourth leading cause of disability. Recent researchers are working on alternative therapies for the treatment of depression to prevent unwanted adverse drug reactions and improve efficacy. Chemical constituents of various herbs provide an extensive research area for the treatment of depression. This study aims to highlight the efficacy of phytochemicals in depression. Metabolites of various plants from diverse classes, such as polyphenols (flavonoids, lignanes, phenolic acids and coumarins), terpenes and terpenoids, alkaloids, saponins and sapogenins, carbohydrates and different amines possessed antidepressant activity (Bahramsoltani et al., 2015).

Different types of synthetic antidepressant chemicals were established and introduced, for example, SSRI (selective serotonin reuptake inhibitors) and TCA (tricyclic antidepressants). The pharmacological effect of antidepressants has various side effects, like psychomotor impairment, addiction, and dependency (Sarko, 2000). Many researchers investigated various medicines for the management of depression, in which investigation on numerous herbal-based medicines has contributed to significant pharmacological uses (Xiong JY et al., 2007).

Pulsatilla nigricans belongs to the buttercup family (Ranunculaceae). P. nigricans is indigenous to Turkey, Germany, Russia, Sweden, Southern England, France, Denmark, and Asia (Felter HW, Lloyd JU., 1983). The flower of Pulsatilla nigricans is dark violet-brown, and the whole plant is covered with white, soft velvety hairs (Kumar et al., 2008). The principal components of Pulsatilla nigricans are anemonic acid, potassium sulfate and oil of anemone, anemone camphor, isoanemonic acid, anemonin and saponin, (Dr. Sudheera. A P, 2012).

Because of increasing interest in plant-based treatments for psychiatric disorders, particularly depression, *Pulsatilla nigricans* presents a promising candidate for further exploration. Its traditional use in relieving anxiety, restlessness, and nervous disorders suggests it may influence neurochemical pathways involved in depression. Additionally, its nutritional profile supports overall brain function by contributing to cellular protection and neurotransmitter balance. Therefore, based on its therapeutic applications and phytochemical composition with potential neuroactive properties, the current study was designed to investigate the antidepressant effects of *Pulsatilla nigricans* through validated animal models of depression. This research aims to provide scientific evidence supporting its traditional use and to identify its potential as a natural antidepressant agent.

Materials and methods

Study Protocols were approved by the Animal Ethical Committee, Hamdard University. P. nigricans was purchased via Mektum Homoeo Pharma, Pakistan. For extraction purposes, 2 kg plant dried and crushed plant leaves were soaked for about 7 days in 70% ethanol and filtered. Filtrated extract of Pulsatilla nigricans was dried at 30-40°C, with the help of a Rotary Evaporator (Buchi B-169 Vacuum system, Switzerland) and stored in an airtight container.

Wistar rats of about 150-250 g of either sex were procured from Hamdard University (Animal House). Animals were placed in well-ventilated, eco-friendly conditions and temperatures and allowed free access to food and water. All the experimentations were performed as per endorsed procedures specified by the Animal Ethics Committee.

Wister rats of weight 200 – 250 g were grouped (4 groups), containing seven rats each. Group 1 (control) and 2 (standard), provided with vehicle (normal saline) and fluoxetine via oral route (20 mg/kg). Whereas groups 3 and 4 were labeled as treated, and kept on EEPN administered via oral at 250 and 500 mg/kg doses.

Grouping and Dosing Protocol

Table 1. Grouping And Dosing Protocol of Behavioral Screening

Groups	Control	Standard	Treated group I	Treated group II
No. of animals	n = 07	n = 07	n = 07	n = 07
Treatment	Normal saline	Fluoxetine	EEPN I	EEPN II
Dose	10 ml/kg	20 mg/kg	250 mg/kg	500 mg/kg

EEPN = Ethanolic extract of Pulsatilla nigricans

The following two models were used for antidepressant activity

(i) Forced Swim Paradigm

Forced swim paradigm, conducted as per the method proposed by Porsolt et al., 1977. The equipment comprises a plastic cylinder loaded with water (maintaining temperature at 25 ^{0}C), which helps to induce stress. Antidepressant agents decrease immobility time. Randomly grouped rats received the drug treatment and control as per the protocol mentioned above. Initially, a pretest of two swim sessions for an initial 15 min was conducted, and at least 24 h later, rats were placed again in the container for up to 5 min, in which the duration of immobility time was noted, and before returning to the home cage, all rats were dried properly.

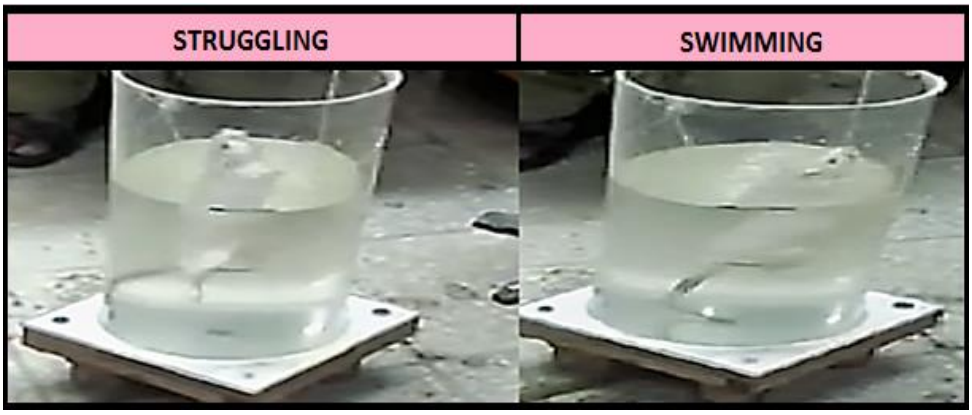

Figure 1: Forced Swim Test

(ii) Tail Suspension Paradigm

Tail suspension paradigm, conducted as per the method proposed by Steru et al. 1985. All the rats were kept for 1 h before the behavioral procedure in the experimental room to acclimatize. The experimental model was performed 45 min after administration of EEPN, fluoxetine, and vehicle to respective groups as described above in the protocol, rats of all groups were suspended one by one from a horizontal surface via the tail by using tape placed 1 cm from the tip of the tail. After 1 min of acclimatization, immobility time was noted for 5 min. Animals were only considered immobile when they were static and suspended inactively.

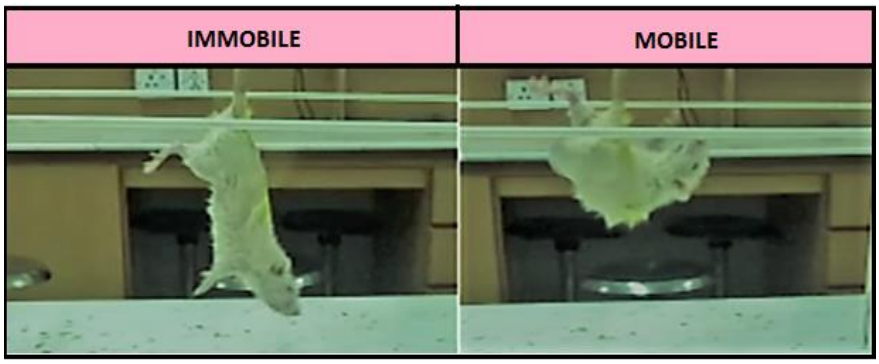

Figure 2: Tail Suspension Test

The results have been reported as mean ± S.E.M. The standard and treated groups were assessed with a control group by applying one-way ANOVA with Tukey HSD post hoc analysis. The results were assumed significant at $p<0.05$.

Results

Table 2 illustrates the effects of EEPN. In which the treated groups (EEPN 250 mg/kg and 500 mg/kg) and standard group (fluoxetine) produce significant decreases in the time of immobility as compared to the control group.

Table 2. Effect of EEPN on immobility time in FSP

GROUPS	IMMOBILITY TIME (min)
Control	85.7 + 2.4
Standard	57.8 + 7.3*
EEPN 250 mg/kg	62.8 + 3.3*
EEPN 500 mg/kg	63.0 + 5.6*

Shows Values are mean ± SEM (n=7), *P < 0.05 significant as compared to control. EEPN = Ethanolic extract of Pulsatilla nigricans.

Table 3 illustrates the effects of EEPN on TSP, according to which a significant decrease in immobility time duration was noted in animals of the treated groups (EEPN 250 and 500 mg/kg) and standard groups (fluoxetine) as compared to animals of the control group.

Table 3. Effect of EEPN on immobility time in TSP

GROUPS	IMMOBILITY TIME (min)
Control	94.8 + 1.6
Standard	68.1 + 7.6*
EEPN 250 mg/kg	74.8 + 3.3*
EEPN 500 mg/kg	75.0 + 5.6*

Shows values are mean ± SEM (n=7), *P < 0.05 significant as compared to control. EEPN = Ethanolic extract of Pulsatilla nigricans.

Discussion

To estimate antidepressant activity in the current investigation, fluoxetine was used as the standard. It is SSRIs that selectively increase serotonin levels in the brain (Wang et al., 2008). Physicians commonly use classical antidepressant agents, i.e. 5HT, TCAs, and MAOIs, but some of these agents produce toxicity, due to which SSRIs are most frequently used (Feighner JP., 1999).

In this study, it was noticed that EEPN 250 and 500 mg/kg exhibited significant anti-depressant effects. In the forced swim test and tail suspension test, EEPN and fluoxetine considerably decreased immobility time as compared to the control. Immobility is the state of hopelessness when rats are allowed to swim in a narrowed or limited area or are suspended by the tail so that which they cannot run away easily. This proposes disillusionment to continue in escape after persistent stress or develop passive behavior to cope with a stressful environment (Lucki I., 1997). A decrease in immobility time signifies that the state of depression is also decreased (Porsolt RD, Anton G, Blavet N, 1978) (Borsini F, Meli A., 1988).

Antidepressant activity of Pulsatilla nigricans is traditionally reported, but may not have any reported model study. According to exciting evidence that anxiety and depression signs and symptoms overlap with each other (Shader RI, Greenblatt DJ., 1995). It is also stated that several anxiolytic agents have been used in the treatment of depression and vice versa (Haefely W., 1992). This suggests that there must be a definite comparable etiology in the progress of depression and anxiety. Furthermore, saponin is among the active components of P. nigricans, and it also possesses an antidepressant effect.

Earlier, several theories have been proposed to explain this feature. In serotonergic theory, it was proposed that biochemical significance and acute and chronic behavior of antidepressant agents can reduce serotonergic neurotransmission, which results in anxiety and depression due to unnecessary functioning of the serotonergic punishment system (Deakin, 1983). There are also several reports relating serotonergic agents as antidepressants and anxiolytics (Yocca, 1990) (Labrid C, Mocaer E, Kamaun A., 1992) (Murphy DL, Mitchell PB, Potter WZ., 1995).

Another hypothesis proposes that GABAergic, which possesses anxiolytic activity, may also produce antidepressant activity (Lloyd KG, Zivkovic B, Scatton B, Morselli PL, Bartholoni G., 1989). It has also been reported that several GABA mimetic drugs are also effective as antidepressants (Lloyd KG, Zivkovic B, Scatton B, Morselli PL, Bartholoni G., 1989). All these studies reveal that anxiety and depression may have some mutual etiological factors additionally, those drugs which exhibit both antidepressant activities should be studied further for their beneficial uses.

Consequently, the antidepressant results of this study show that there may be some common aspects associated with the anxiolytic effect of Pulsatilla nigricans, which contribute to its antidepressant effect as well.

Conclusion

In the current study, Pulsatilla nigricans was found to have antidepressant effects, which were tested using two different methods: the FST and TST. These methods are normally used to determine depressive behavior in rats. The plant, which is typically used in homoeopathic treatments, has now been shown scientifically to have antidepressant properties in rat models. However, the study suggests that more research is needed to understand how exactly the plant works to produce these effects.

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Conflict of interest

The author has no conflict of interest

Authors' contributions

RS is a chief investigator, IY directed the research work, and SM executed statistical analysis. NA and TBF helped in writing the manuscript, and TM assisted in compiling the data.

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