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Antidepressant activity evaluation of *Actaea spicata* L. Roots

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Abstract

*Actaea spicata* L. (Ranunculaceae) has long tradition of use as antidepressant but no systematic phytochemical and pharmacological work has been ever carried out to validate its traditional claims. Thus, the present investigations have been envisaged to evaluate antidepressant potential of *A. spicata*. Petroleum ether (60-80°C), chloroform, methanol and water extracts of *A. spicata* were evaluated for antidepressant activity in mice using despair swim test at dose levels of 100, 200 or 400 mg/kg, p.o. Amongst various extracts tested, only methanol extract exhibited significant antidepressant activity at a dose of 200 mg/kg. Preliminary phytochemical screening showed presence of alkaloids, flavonoids and triterpenoids in methanol extract of *A. spicata*. The present investigation validated traditional claims of the plant. It is finally concluded that antidepressant activity of *A. spicata* may be attributed to flavonoids.

Keywords: *Actaea spicata*, Alkaloids, Antidepressant and Flavonoids

Introduction

According to the World Health Organization, depression is a medical and social problem affecting 340 million people worldwide. The lifetime prevalence of depression is as high as 20% in the general population worldwide with a female to male ratio of about 5:2. Typically, the course of the disease is recurrent and most patients who recover from major depressive episodes still become depressed afterwards. The synthetic drugs are frequently prescribed especially tricyclic antidepressants for the treatment of depression. Though efficacious for the treatment of depression, these antidepressant drugs
frequently produce side effects; for instance, dry mouth, mydriasis, constipation, sleepiness, temporary fatigue, restlessness and headaches. Often, the consequence of antidepressant-induced sexual dysfunction is observed on discontinuation of the antidepressant treatment. Thus, researchers are exploring natural resources for newer, safer and efficacious antidepressant drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable and cost effective strategy to develop new drugs.

*Actaea spicata* has been traditionally used in the treatment of rheumatism, inflammation, rheumatic fever, lumbago, scrofula, depression, nervous disorders, chorea, and as emetic, expectorant, laxative, stomachic and purgative. Isoquinoline alkaloids and triterpene glucosides have been isolated from *A. spicata*.

Despite a long tradition use of *A. spicata* as antidepressant, no scientific work has ever been carried out to justify its traditional claims for antidepressant activity. Therefore, it was envisaged to evaluate various extracts viz., petroleum ether (60-80°C), chloroform, methanol, water of *A. spicata* for antidepressant activity in mice.

**Materials and Methods**

**Plant material**

*A. spicata* roots were procured from K.R. Indo German American Trading Company, Kurukshetra (Haryana), India. The identity of the plant was confirmed through Dr. H.B. Singh, Scientist F, Head of Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India.

**Preparation of A. spicata extracts**

Petroleum ether (60°–80°C), chloroform (Ranbaxy Laboratory Chemicals) and methanol (S.D. Fine Chemicals), all of LR grade, were used for extraction of the plant material. Dried coarsely powdered *A. spicata* roots (800 g) were successively Soxhlet extracted with petroleum ether (60-80°C), chloroform and methanol. The marc was air dried, and water extract was obtained by boiling with distilled water for 2 h. Solvents from extracts were recovered under reduced pressure using rotary vacuum evaporator (BUCHI, Switzerland), and the dried extracts were preserved in a vacuum desiccator containing fused calcium chloride (S.D. Fine Chemicals).

**Phytochemical screening**

Specific standard reagents were used for screening various extracts of *A. spicata* for different classes of phytoconstituents.

**Pharmacological studies**

**Experimental animals**

Male Laca mice (20-24g) were used in the entire study. The animals were fed standard pellet diet (Ashirwad, Chandigarh) and water *ad libitum*. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 h light-dark cycle. Groups of five mice were used in all sets of experiments. The animals were fasted for 18 h before use. The approval from the Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences and Drug Research, Patiala was taken before caring out biological studies.
**Vehicle and standard drug**
Distilled water + Tween 80 (5%) was used as vehicle for preparing test doses of different extracts of *Actaea spicata*. Imipramine (Triko Pharmaceuticals, Rohtak, Haryana) was used as a standard drug. The antidepressant effects were evaluated after acute oral administration of test substances.

**Experimental protocol**
The protocol designed to perform present investigation has been depicted in table 1. This table depicts experimental model used, treatments, doses and route of administration, and number of animals used.

**Despair swim test**
Mice were allowed to swim, after 1h of administration of test substances in a plexiglas cylinder (height 40 cm and diameter 18 cm) filled with water upto the level of 15 cm, and maintained at 25 ± 2°C for 6 min. During this test period, the total duration of immobility (floating in the water in a slightly hunched but upright position, its nose above the surface) was noted.

**Statistics**
The results have been expressed as mean ± standard deviation (S.D.). The test doses were compared with standard and control by one way analysis of variance (ANOVA) followed by Student Newman Keul’s test.

**Results**
Various extracts viz., petroleum ether, chloroform, methanol and water extracts of *Actaea spicata* roots were prepared successively in a Soxhlet apparatus. The yields of various extracts viz., petroleum ether, chloroform, methanol and water of *Actaea spicata* are shown in table 2. All the extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents. Petroleum ether extract gave positive tests for the presence of lipids; chloroform extract for alkaloids; methanol extract for alkaloids, flavonoids, triterpenoids, tannins, carbohydrates and proteins; and water extract for tannins, carbohydrates and proteins. These extracts were evaluated for antidepressant activity at various dose levels, i.e., 100, 200 or 400 mg/kg, p.o. in mice. The activity was compared with that observed in the control group as well as with the group treated with the standard antidepressant drug imipramine (10 mg/kg). The time spent by the mice in immobile state after oral administration of various doses of the extracts of *Actaea spicata* roots, control and standard drug has been shown in table 3. Among various extracts of *Actaea spicata* tested, significant antidepressant activity was observed in the methanol extract at a dose of 200 mg/kg with respect to control. The methanol extract (200 mg/kg) exhibited statistically equivalent antidepressant activity as exhibited by imipramine. Petroleum ether, chloroform and water extracts of *Actaea spicata* roots were found to be devoid of antidepressant activity.

**Discussion**
Antidepressant activity of various extracts of *Actaea spicata* roots was evaluated employing a widely used model, i.e., Despair swimming test. The model was chosen since it is effective, cheap, simple, less time consuming, and requires no preliminary training to the rats and does not cause much discomfort to the animals while handling. The model is principally based on the observations that rats forced to swim in a restricted space from which they cannot escape induce to characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. The ultimate manifestation of depression in the animals are exhibited by decrease in motor activity, which is measured by the time spent by the animal in immobile state.
Dried petroleum ether, chloroform, methanol and water extracts of *Actaea spicata* roots, were prepared by successive extraction in a Soxhlet apparatus, and were evaluated for antidepressant activity test at various dose levels, i.e., 100, 200 or 400 mg/kg, p.o. The methanol extract significantly reduced time spent by the
mice in immobile state at a dose of 200 mg/kg, thus, exhibited antidepressant activity with respect to control. None of other extracts of *A. spicata* roots possessed antidepressant activity. Preliminary phytochemical studies have shown presence of alkaloids, flavonoids and triterpenoids as major constituents in methanol extract of *A. spicata* roots. Thus, it is suggested that these constituents may be responsible for antidepressant activity of the plant. Amongst these groups of phytoconstituents, flavonoids have been considered main constituents which possess CNS activities. Even recent studies have shown antidepressant activity in flavonoids obtained from natural products. A flavonoid, rutin, obtained from leaves of *Schinus molle* showed potent antidepressant activity in forced swimming test and tail suspension test\(^1\). Rutin (0.3-3mg/kg p.o) showed significant increase in availability of serotonin and nor adrenaline in synaptic cleft, thus exhibiting antidepressant effect. Liquiritin exerted antidepressant activity in forced swimming and tail suspension tests at the dose level of 20 mg/kg p.o. by increasing noradrenaline and 5HT levels in hippocampus, cortex and hypothalamus\(^2\). The defence of liquiritin against oxidative stress at a dose of 20 mg/kg p.o. showed significant results in CUMS-induced depressed rats\(^3\). Hypericin, (0.2 mg/kg) hyperoside (0.6 mg/kg), isoquercitrin (0.6 mg/kg) and miquelianin (0.6 mg/kg) exhibited antidepressant effect by reducing HPA (hypothalamic- pituitary adrenal) axis function in rats\(^4\).

The present investigations validated the traditional claims of *A. spicata* as antidepressant drug. Further, it is concluded that antidepressant activity of *A. spicata* roots may be attributed to flavonoids, and the probable mechanisms may involve various neurotransmitters.

**References:**


