Neuropharmacological investigations on
*Actaea acuminata* Wall. ex Royle roots

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*Actaea acuminata* Wall. ex Royle, synonym of *Actaea spicata* var. *acuminata* (Wall. ex Royle) H.Hara, commonly called the Himalayan Baneberry (*Ranunculaceae*) has been investigated for various pharmacological activities, based on its traditional claims. Properly identified *A. acuminata* roots were defatted by extracting with petroleum ether. The marc was then extracted in a Soxhlet apparatus with methanol. Various pharmacological activities such as antianxiety (Elevated plus maze, Hole board and Light/Dark tests), anticonvulsant (Maximum electroshock test), antidepressant (Despair swim test), sedative (Actophotometer), antistress (Cold swim test), analgesic (Tail immersion test) and anti-inflammatory (Carrageenin-induced paw edema model) were evaluated after administration of 50, 100 or 200 mg/kg, *p.o.*, doses of methanol extract. The methanol extract exhibited significant antianxiety, anticonvulsant, antidepressant and antistress activities, and mild sedative activity at a dose of 200 mg/kg. It was found to be devoid of analgesic and anti-inflammatory activities. Preliminary phytochemical screening of methanol extract showed the presence of alkaloids and polyphenols. Thus, CNS activities of the plant may be attributed to these groups of phytoconstituents.

**Keywords:** Antianxiety, Anticonvulsant, Antidepressant, Antistress, CNS activity, Himalayan Baneberry, *Ranunculaceae*, Sedative

Mental disorders, particularly anxiety and depression, have registered an alarming rise during the last two decades gradually over taking the incidence of the microbial diseases affecting more than 650 million people worldwide. Serious neurological and behavioural disorders make up 13% of the global disease burden surpassing both cardiovascular diseases and cancer. About 20% of the world’s children and adolescents suffer from mental disorders, and about 900,000 people commit suicide every year. In US, 61.5 million adults experience mental illness and 13.6 million suffer with serious mental illness such as schizophrenia, major depression or bipolar disorder. In India, 6-7% of the population suffer from a mental disorder.

The emergence of mental disorders has attracted the attention of researchers towards various pharmacotherapeutic approaches for the management of these ‘modernization borne diseases’. Regular use of synthetic drugs causes deterioration of cognitive functioning, addiction, physical dependence and tolerance. Abrupt cessation of chronic treatment with benzodiazepines causes appearance of withdrawal effects comprising rebound anxiety, restlessness, epilepsy, and motor agitation. In the light of adverse effects associated with the synthetic drugs, natural resources have gained importance among researchers looking for safer and effective 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 acuminata* Wall. ex Royle, synonym of *Actaea spicata* var. *acuminata* (Wall. ex Royle) H.Hara from *Ranunculaceae*, commonly known as Himalayan Baneberry, is one such plants which has long tradition of use in the treatment of nervous disorders but has not been investigated systematically.

A survey of ethnopharmacologic records reveals that the plant has been traditionally used in the treatment of rheumatism, inflammation, rheumatic fever, lumbago, scrofula, nervous disorders, chorea, and as emetic, expectorant, laxative, stomachic and purgative. In folk medicine, roots are used to treat ovarian neuralgia, uterine tenderness and subinvolution. The berries of *A. acuminata* are used internally for asthma and scrofula, and externally for skin diseases. In traditional health care system of
Uttarakhand, decoction of *A. acuminata* roots is used in the treatment of bronchial asthma; the root powder is given to children for cough, and leaves to treat sciatica. In the present study, we tried to validate the traditional claims of *A. acuminata*, and explored its roots for various pharmacological activities.

**Materials and Methods**

**Plant material**

Dried roots of *A. acuminata* were procured from Himalaya Herbs Store, Saharanpur, Uttar Pradesh, India in September, 2008. The identity of the plant was confirmed by Dr. HB Singh, Scientist F, Raw Material Herbarium and Museum (RHMD), CSIR-National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref. No. NISCAIR/RHMD/Consult/-2008-09/1170/202).

**Animals**

Sprague Dawley rats of body weight 250-300 g, either sex, purchased from the CSIR-Institute of Microbial Technology, Chandigarh, India were used for pharmacological and toxicological studies. The animals were fed with normal laboratory pellet diet and water ad libitum. The approval was taken from Institutional Animal Ethics Committee of Punjabi University, Patiala before carrying out animal studies (107/99/CPCSEA/2012-25, dated 02/10/2012). The animals were acclimatized to the Pharmacognosy laboratory, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, daily for 1 h for 7 continuous days before the start of experiment. After acclimatization, the animals were kept in the Central Animal House, Punjabi University, Patiala. All the experiments were carried out from 9 AM to 12 PM as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals. Groups of six animals were used in all sets of experiments. The animals were fasted overnight before use. The doses were administered orally with the help of an oral cannula fitted on a tuberculin syringe.

**Solvents**

Methanol (S.D. Fine Chemicals, Mumbai, India) and petroleum ether (60-80°C) (RFCL Ltd., New Delhi, India), of LR grade, distilled under normal atmospheric pressure, were employed.

**Chemicals and instruments**

Rotary vacuum evaporator (BUCHI, Switzerland) was used for recovery of solvent under reduced pressure. Digital actophotometer, plethysmometer (INCO, Ambala, India) and electroconvulsiometer (Rolex, Patiala, India) were used for sedative, anti-inflammatory and anticonvulsant activities respectively.

**Preparation of methanol extract of *A. acuminata* roots**

Dried coarsely powdered *A. acuminata* roots (1 kg) were successively and exhaustively Soxhlet extracted using solvents in order of increasing polarity viz., petroleum ether (60-80°C) and methanol. Solvents from extracts were recovered under reduced pressure using rotary vacuum evaporator. Dried methanol extract was preserved in a vacuum desiccator containing fused calcium chloride.

**Vehicle and Standard**

Distilled water + Tween 80 (2%) was used as vehicle for preparing various test doses of methanol extract and standard drugs in such a concentration as to administer a volume ranging 1-2 mL to the animals. Diazepam @ 1, 2 and 5 mg/kg, p.o., phenytoin sodium injection (100 mg/kg, i.p.), imipramine (5 mg/kg, p.o.), indomethacin (5 mg/kg, p.o.) and pentazocine (10 mg/kg, p.o.) were used as standard antistress, anxiolytic, sedative, anticonvulsant, antidepressant, anti-inflammatory and analgesic drugs respectively. Diazepam, imipramine and indomethacin were collected as gift samples from Triko Pharmaceuticals, Rohtak, Haryana for research purpose only. Phenytoin sodium injection (Magnet Labs Pvt. Ltd., New Delhi, India) and pentazocine tablets (Ranbaxy, Gujrat, India) were purchased from local market.

**Experimental protocol**

Animals were divided into five (I-V) groups. Group I, Control group received vehicle (1 mL, p.o.); Group II, Standard group received respective standard drug; Group III-V, Test groups received methanol extract (50, 100 and 200 mg/kg, p.o.).

**Statistics**

The results have been expressed as mean ± SD. The test drugs were compared with standard drug and control by one way analysis of variance (ANOVA) followed by Student Newman Keul’s test.

**Evaluation of antianxiety activity**

*Elevated plus maze model (EPM)*

The plus-maze apparatus consisting of two open arms (50×10 cm) and two closed arms (50×10×40 cm)
having an open roof, with the plus-maze elevated (48 cm) from the floor was used to observe anxiolytic behavior in animals\textsuperscript{15}. Each rat was placed at the centre of the elevated plus maze with its head facing the open arms. During the 5 min experiment, the behavior of the rat was recorded as: (A) the number of entries into the open arms; and (B) average time spent by the rat in the open arms (average time = total time spent in open arms/number of entries in arms). Test substance was administered orally using a tuberculin syringe fitted with oral cannula. Dose administration schedule was so adjusted that each rat was having its turn on the EPM apparatus, 45 min after the administration of the dose.

**Light/Dark test**

It consists of open top wooden box. Two distinct chambers, a black chamber (25×35×35 cm), painted black and made dark by covering its top with black plywood, and a bright chamber (25×35×35 cm), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. The two chambers were connected through a small open doorway (7.5×5 cm) situated on the floor level at the centre of the partition\textsuperscript{16}.

Thirty min after the administration of the test drug, each rat was individually placed in the centre of the light compartment (facing away from the door). During 5 min test period, latency of the first crossing from light to dark compartment and mean time spent in light zone were noted.

**Hole board test**

A square test arena (60×60 cm) with four holes (4 cm in diameter) on the surface was placed 23 cm above the floor. Thirty min after the administration of the test drug, each rat was individually placed in the centre of the board (facing away from the observer). During 5 min test period, number of head dips was noted\textsuperscript{115}.

**Evaluation of antidepressant activity**

**Despair swim test**

Rats were forced to swim, after 1 h of administration of test substances, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water up to the level of 15 cm, and maintained at 25±2°C\textsuperscript{18}. Rats were allowed to swim 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.

**Evaluation of antistress activity**

**Cold swimming test**

Rats were forced to swim, after 1 h of administration of test drug, in a Plexiglas cylinder (height 40 cm; diameter 18 cm) containing water up to the level of 15 cm, and maintained at 10±2°C\textsuperscript{19}. Rats were allowed to swim for 6 min. During this test period, the total duration of immobility was noted.

**Evaluation of sedative activity**

**Actophotometer**

Spontaneous locomotor activity was measured using a digital actophotometer (24×22×10 cm) with automatic counting of animal movements on the activity cage floor. Rats were placed individually in activity cage for 10 min test period, 30 min after administration of test drug. The number of line crossings by the animal due to beam interruptions before and after treatments were recorded. The count corresponds to locomotor activity\textsuperscript{20}. Percentage reduction in activity was also noted.

**Evaluation of analgesic activity**

**Tail immersion test**

Groups of rats were subjected to noxious stimulus (radiant heat) by placing 5 cm of the tail in a 500 ml beaker containing 450 ml water maintained at 55±2°C before and after treatment with test drug\textsuperscript{21}. The tail withdrawal from the heat (flicking response) was taken as end point. A cut off period 15 s was observed to prevent damage to the tail. Three basal reaction times for each rat at a gap of 5 min were taken to confirm normal behaviour of the rat. The reaction time at 30 min, 1, 2, 3 and 6 h were recorded after the treatment. The percentage maximum possible effect (% MPE) was calculated from the formula as given below:
\[
\% \text{MPE} = \frac{\text{Reaction time} - \text{basal time}}{\text{Cut off time} - \text{basal time}} \times 100
\]

**Evaluation of anti-inflammatory activity**

*Carrageenin induced paw edema model*

All rats were weighed and marked for identification. A mark was made on both the hind paws (left and right) of each rat at the level of the lateral malleolus, so that every time the paw could be immersed in the solution up to the fixed mark to ensure accurate reading of the paw volume. The initial paw volume of each rat before treatment was noted by means of volume displacement method using a plethysmometer. After 1 h of treatment, 0.1 ml of 1% carrageenin solution in normal saline was injected in the plantar region of the left hind paw of each rat. Paw volume of each rat was measured using a plethysmometer after the carrageenin injection at 1, 2 and 3 h intervals. Percent inhibition of paw edema was calculated using the given formula and was expressed as the extent of anti-inflammatory activity shown by various test samples.

\[
\% \text{Inhibition of paw edema} = \frac{(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}}{(V_t - V_0)_{\text{control}}} \times 100
\]

\(V_0\) = volume of the paw before treatment and \(V_t\) = volume of paw after carrageenin injection at 1, 2 and 3 h.

**Results and Discussion**

**Antianxiety activity of methanol extract of *A. acuminata* roots**

The methanol extract of *A. acuminata* roots was subjected to antianxiety activity in rats using elevated plus maze (EPM), light/dark and hole board tests. The number of entries and average time spent in open arms using EPM model, latency to leave light chamber and average time spent in light chamber using Light/Dark model, and average number of dips using hole board test in the rats after oral administration of 50, 100 or 200 mg/kg of methanol extract of *A. acuminata* roots, diazepam (2 mg/kg) and the control (vehicle) have been shown in Fig. 1 (A-C, respectively). Amongst various doses tested, only 200 mg/kg dose of the methanol extract exhibited significant antianxiety activity which was at par with that of standard drug, diazepam (2 mg/kg) in all experimental models of anxiety. The methanol extract possessed significant antianxiety activity at 100 mg/kg with respect to control but the activity was...
not equivalent to the standard drug, whereas 50 mg/kg dose did not show antianxiety activity.

**Anticonvulsant activity of A. acuminata root extract**

The methanol extract of *A. acuminata* roots was subjected to anticonvulsant activity in rats using MES test. The decreased duration of MES-induced tonic extension phase in the rats and percentage protection of animals were noted after administration of 50, 100 or 200 mg/kg, p.o. doses of *A. acuminata* roots extract, phenytoin (100 mg/kg, i.p.) and the control (vehicle, p.o.). Methanol extract exhibited significant anticonvulsant activity at the dose of 200 mg/kg, p.o. which was equivalent to the standard drug, phenytoin (100 mg/kg). At lower dose, i.e., 100 mg/kg, mild anticonvulsant activity was observed as it did not achieve therapeutic level whereas 50 mg/kg dose did not possess anticonvulsant activity. Fig. 2 shows anticonvulsant activity of methanol extract of *A. acuminata* roots.

**Antidepressant activity A. acuminata root extract**

The methanol extract of *A. acuminata* roots was subjected to antidepressant activity in rats using despair swim test. The mean immobility time of the rats after oral administration of 50, 100 or 200 mg/kg doses of *A. acuminata* roots extract, imipramine (5 mg/kg) and the control (vehicle) has been shown in Fig. 3A. The methanol extract exhibited significant antidepressant activity at the dose of 200 mg/kg, p.o. which was similar to standard drug, imipramine. At 100 mg/kg dose, the methanol extract exhibited significant antidepressant activity with respect to control but the activity was not equivalent to the standard drug. The methanol extract was found to be devoid of antidepressant activity at the dose of 50 mg/kg.

**Antistress activity of methanol extract of A. acuminata roots**

The methanol extract of *A. acuminata* roots was subjected to antistress activity in rats using cold swim test. The mean immobility time of the rats after oral administration of methanol extract of *A. acuminata* roots (50, 100 or 200 mg/kg), diazepam (1 mg/kg) and the control (vehicle) has been shown in Fig. 3B. The methanol extract significantly reduced mean time spent by rats in immobile state at the dose of 200 mg/kg, p.o. in comparison to control. At lower doses, i.e., 50 or 100 mg/kg, the methanol extract could not achieve therapeutic level when compared to standard drug.

**Sedative activity of methanol extract of A. acuminata roots**

The methanol extract of *A. acuminata* roots was subjected to sedative activity in rats using actophotometer. The number of line crossing by the animal due to beam interruptions in actophotometer...
after administration of methanol extract of *A. acuminata* roots (50, 100 or 200 mg/kg, p.o.), diazepam (5 mg/kg, p.o.) and the control (vehicle) has been shown in Fig. 4. The standard drug reduced motor activity in rats to 55.96% with respect to control where as methanol extract at the dose of 200 mg/kg reduced motor activity to 19.17%, inferring its mild sedative activity.

**Analgesic activity of methanol extract of* A. acuminata *roots**

The methanol extract of *A. acuminata* roots was subjected to analgesic activity in rats using tail immersion method. The tail withdrawal from the heat (flicking response) recorded in the rats after oral administration of 50, 100 or 200 mg/kg doses of *A. acuminata* roots extract, pentazocine (10 mg/kg, p.o.) and the control (vehicle) has been shown in Fig. 5. The methanol extract did not exhibit analgesic activity at any of the tested dose during 6 h of observation as it did not significantly increase pain threshold in terms of reaction time. The percent maximum possible effect observed in animals treated with methanol extract at different doses is significantly less than that shown by animals treated with standard drug during 6 h of study recorded at 30 min, 1, 2, 3 and 6 h.

**Anti-inflammatory activity of methanol extract of* A. acuminata *roots**

The methanol extract of *A. acuminata* roots was subjected to anti-inflammatory activity in rats using carrageenin-induced paw edema model. The increase in paw volume and % inhibition of paw edema of the rats after oral administration of 50, 100 or 200 mg/kg doses of *A. acuminata* roots extract, indomethacin (5 mg/kg) and the control (vehicle) has been shown in Fig. 6. The methanol extract did not exhibit activity at any of the tested dose during 3 h of observation as it...
did not show significant percent inhibition of paw edema in rats with respect to control.

The well established experimental models were employed for assessment of anxiolytic potential of methanol extract of *A. acuminata* roots. The fear due to height (acrophobia), bright illumination and novel environment induce anxiety in the animals when placed on the EPM, light/dark and hole board apparatus respectively. The ultimate manifestation of anxiety in the animals are exhibited by decreased motor activity measured by number of entries and average time spent in the open arms of EPM, latency to leave light chamber and time spent in the light chamber of light/dark apparatus, and the number of head dips in hole board apparatus by the animal. Our results confirm significant antianxiety activity of methanol extract of *A. acuminata* roots at the dose of 200 mg/kg, p.o as it significantly increased all parameters assessed compared to control. Anticonvulsant activity of methanol extract of *A. acuminata* roots was evaluated employing MES test. In this test, rats receive an electrical stimulus of sufficient intensity to induce maximal seizures of their hind limbs, with tonic extension as the endpoint of the test. The methanol extract significantly inhibited tonic extension in rats, suggesting its anticonvulsant activity against generalized tonic-clonic and cortical focal seizures.

Antidepressant and antistress activities of methanol extract of *A. acuminata* roots were evaluated employing despair swim and cold swim tests, respectively. These models are principally based on the observations that rats forced to swim in a restricted space from which they cannot escape induce a 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. When rats are individually kept in a cold environment leads to a sharp increase in the level of adrenocorticoids. This increased level of neurotransmitter induces stress in animals. The methanol extract significantly reduced mean time spent by the animals in immobile state as compared to control group, thus, inferring its antidepressant and antistress activities. Sedative activity of methanol extract of *A. acuminata* roots was evaluated using actophotometer apparatus. The activity of test drugs was assessed by recording number of line crossing by the treated animal due to beam interruptions. The decrease in activity scores after treatment correspond the sedative activity in experimental rats. Our results suggest mild sedative activity of methanol extract in tail flick model, and the analgesic activity of the test drug in different doses is assessed in terms of significant increase in pain threshold during the period of observation, and this indicates the involvement of a higher centre. Carrageenin-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenin is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effects. Moreover, the experimental model exhibits a higher degree of reproducibility. Carrageenin induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas second phase is related to the release of prostaglandins and slow reacting substances which peak at 3 h. The methanol extract of *A. acuminata* was found to be devoid of analgesic and anti-inflammatory activities.

Several neurotransmitters namely serotonin, norepinephrine, γ-aminobutyric acid (GABA), that are produced in the brain, directly influence one’s feelings about a given situation. These brain neurotransmitters and hormones levels change immediately with neurological disorders. In particular, monoamines, such as serotonin, norepinephrine and dopamine, are involved in mood, stress and other physical homeostasis. Various modes of action for neuropharmacological activities of methanol extract of *A. acuminata* roots are suggested. The γ-aminobutyric acid type A (GABA_A) receptor, the chloride ion channel complex, and the central benzodiazepine receptors located on the neuronal membrane with in this complex have been suggested to play an important role in the regulation of stress, anxiety, and insomnia. The 5-hydroxytryptamine 1A (5-HT_1A) receptor also plays an important role in psychiatric disorders, notably anxiety and depression. Monoamine inhibitory activity (tribulin activity) is associated with conditions associated with stress and anxiety, both in animals and in human beings. In brain, nitric oxide synthase (NOS) has been localized in regions involved with anxiety, such as hypothalamus, amygdala and hippocampus. Inhibition of NOS by nonselective or by relatively selective inhibitors of nNOS produced antianxiety like effects.

Preliminary phytochemical studies showed the presence of alkaloids and polyphenols as major
groups of phytoconstituents in methanol extract of 
*A. acuminata* roots. The available literature reveals 
that a large number of alkaloids—berberine \(^{3,34}\), 
trigonelline \(^{35}\), theacrine \(^{36}\), nicotine, mecaminylamine \(^{37}\), 
morphine \(^{38}\), alstonine \(^{39}\) and erythrosine \(^{40}\) and 
flavonoids—chrysin \(^{41}\), apigenin \(^{42,43}\), linarin \(^{44}\) and 
goodyerin \(^{45}\) have been reported to exhibit varied 
neuropharmacological activities. In agreement to 
these reports, it is suggested from our results that 
neuropharmacological activities of *A. acuminata* roots 
are attributed to flavonoids and/or alkaloids.

**Conclusion**

With the results of the present study, it can be 
concluded that the traditional claims of *A. acuminata* 
roots with relevance to influencing CNS activities 
stands scientifically validated. Detailed investigations 
are in progress to isolate bioactive constituent(s) from 
methanol extract of *A. acuminata* roots responsible 
for neuropharmacological activities.

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