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Sedative, antiepileptic and antipsychotic effects of *Viscum album* L. (Loranthaceae) in mice and rats

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ABSTRACT

Ethnopharmacological relevance: Viscum album L. is claimed in traditional medical practice, to be useful in the treatment of epilepsy and insomnia in Himachal Pradesh, India.
Materials and methods: The effect of *Viscum album* L. on epilepsy, psychosis and sedative activity was evaluated in mice and rats using standard procedure.
Results: The aqueous leaf extract of *Viscum album* L. prolonged the pentobarbital induced sleeping time and reduced the locomotor activity in actophotometer. This suggests that reduced locomotor activity facilitate GABAergic transmission. In addition the extract reduced MES, INH and PTZ-induced convulsions which suggest that there may be possibility of blocking Na^+ channels, opening of Cl^- channels or enhancing the GABAergic system. The extract decreased the apomorphine-induced stereotyped behavior and potentiates the HAL-induced cataleptic score which suggests the extract possess antidopaminergic activity.
Conclusion: The results obtained in present study suggested that title plant exhibited sedative, antiepileptic and antipsychotic activity in mice and rats.

1. Introduction

Viscum album L. is a semiparasitic plant with apparent broad spectrum therapeutic action (Hutt et al., 2001; Eno et al., 2004), belongs to family Loranthaceae. It is also called as mistletoe. Mistletoe refers to a group of perennial flowering plants attached to branches of other trees and shrubs as aerial parasites (Barlow, 1987; Kuijt, 1990). The name 'mistletoe' derives from the Anglo-saxon mistletoe; 'mistel' meaning dung, and 'tan' meaning twig. Thus it literally means 'dung-on-a-twig (Calder, 1983). It is native to Europe and Northern Asian countries and commonly called as "European mistletoe' (Zuber, 2004). *Viscum album* L. is widely used in the folk medicine and traditionally used for the prevention and treatment of several diseases. It is the most widespread species worldwide and has been reputed against cardiovascular diseases, i.e. hypertension and atherosclerosis; various bone and joint disorders including periarthritis, spondylitis, and arthritis; headache; for immune system stimulation; in nervous disorders as sedative or to combat epilepsy (Wichtl and Bisset, 1994; Bartram, 1995; Murray, 1995; Newall et al., 1996). In addition, many ancient polish writers have pen down the use of mistletoe in the treatment of epileptic attacks (Owczarek, 2011). Besides these, it is well known that mistletoe shows effects on the cardiovascular systems by direct action on cholinergic pathways (nervus vagus) responsible for reducing the blood pressure (Wagner et al., 1986). In Himachal Pradesh (northern state of India), the plant is chiefly used to lower blood pressure, to easy anxiety, promote sleep and in treatment of epilepsy. In ethanobotanical uses, the decoction of whole plant is used in enlarged spleen. The plant is used as nutritious fodder for cattle in Kinnaur area, given particularly to calves and goat kids (Praveen and Brij, 2005).

The plant are found to contain various chemical constituents such as lectins (Stirpe et al., 1992; Peumans et al., 1996), viscotoxins (Samuelsson, 1974; Orru et al., 1997), flavonoids (Lorch, 1993; Wollenweber et al., 2000), polysaccharides, biogen amines (Hoffmann, 1990), alkaloids, terpenoids (Deliorman et al., 2001a,b), saponins, tannins, phytosterols, vitamins, hydrocarbons and long-chain fatty acids, etc. (Radenkovic et al., 2006). *Viscum album* L. have been reported to possess antitumors and immunomodulatory (Jurin et al., 1993), anticancer

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(Burger et al., 2001), hypotensive (Ofem et al., 2007), anti-inflammatory (Hegde et al., 2011), hypoglycemic and antioxidant (Orhan et al., 2005), neurophysiological (Radenkovic et al., 2006), cytotoxic (Cebović et al., 2008), antimycobacterial (Deliorman et al., 2001a,b) etc. activities.

In addition there is growing evidence that several herbal preparations such as *Hypericum perforatum* (St. Jhon's wort), *Piper methysticum* (Kava strub), *Ginkgo biloba* (Ginkgo trees) and *Valeriana officinalis* (Valerian) may be efficacious in the treatment of psychiatric disorders (Noel et al., 2008). Antiepileptic and neuropharmacological actions of the plant have not been scientifically studied, the aim of the present study was to evaluate the effects of acute administration of aqueous leaf extract of plant in different models of experimental epilepsy and behavior.

2. Materials and methods

2.1. Preparation of Viscum album L. extract

About a kilogram of fresh leaves of *Viscum album* L. from the host plant (citrus) were collected from a local plantation in Tehri Garhwal (Uttarakhand), during the raining season. The plant was identified and authenticated by Dr. Imran Kazmi, Department of Pharmacognosy, Siddhartha Institute of Pharmacy, Dehradun.

The leaves were first washed free of sands and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the paste (50 g) was weighed and soxhlet extracted with 150 mL distilled water at 100 °C for 8–10 h. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of distilled water. The extract was concentrated to a small volume by rotary evaporator apparatus and then dried at room temperature, corresponding to a 29% yield. Weighed samples of the extract were then used to prepare the stock solution.

2.2. Experimental animals

Swiss albino mice (20–25 g) and Wistar albino rats (150–200 g) of either sex were used for the study. The inbred colonies of mice and rats were obtained and maintained in Siddhartha Institute of Pharmacy, Dehradun, India for experimental purpose. The animals were maintained under controlled conditions of temperature ($23 \pm 2^{\circ}$ C), humidity ($50 \pm 5\%$) and 12 h light–dark cycle (light on from 06:00 to 18:00 h). All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free assessed to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee Siddhartha Institute of Pharmacy, Dehradun, India (1435/PO/a/11/CPCSEA).

2.3. Drug and chemicals

The following drugs were used: Isoniazid (S.d.fine Chemicals); Phenytoin (Zydus Neurosciences, India); Diazepam (Calmpose Inj. Ranbaxy, India); Pentylenetetrazole (Sigma, USA); Pentobarbital sodium (Sigma, USA); Haloperidol (RPG Life Sciences, India); apomorphine sulphate (Sigma, USA); Tween 80 (S.d.fine Chemicals); Ethanol (Sigma, USA).

2.4. Phytochemical analysis

Preliminary phytochemical screening of the crude extract and its aqueous fraction was carried out qualitatively for the presence of alkaloids, glycosides, flavonoids, saponins, sterols, tannins, phenolic compounds, aminoacids, proteins, fatty acids, carbohydrates, volatile oils and terpenes by the following standard methods (Evans, 2005; Khandelwal, 2005).

2.5. Extract standardization by HPLC technique

2.5.1. Preparation of standard and sample solution

A stock solution of betulinic acid was prepared by dissolving 10 mg of standard betulinic acid in 10 mL of methanol (1000 μg/mL) and used as standard. The sample solution was prepared by extracting 1.0 g of dried powdered crude drug with 25 mL of methanol. The methanolic extract was filtered through Whatman filter paper and evaporated to dryness under reduced pressure. The residue obtained was re-dissolved in 1.0 mL of methanol and used for chromatography.

2.5.2. HPTLC instrumentation and procedure

The samples were spotted in the form of bands of width 4.0 mm with a Camag microlitre syringe on precoated silica gel aluminum plate 60F-254 (10 cm × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate 150 nL/s was employed. The slit dimension was kept at 4.0 mm × 0.45 mm and 20 mm/s scanning speed was employed. The mobile phase was composed of toluene:ethyl acetate:acetic acid (5:4:1, v/v/v). The development was carried out in ascending manner in twin trough glass chamber. The optimized chamber saturation time for mobile phase was 20 min at room temperature and the chromatogram was developed up to the length of 80 mm. The developed TLC plates were dried at room temperature with help of an air-dryer. Anisaldehyde in sulfuric acid reagent was used as visualizing agent. The scanning was done in absorbance mode at 570 nm.

2.6. Acute toxicity

The toxicity of extract was studied as per organization for economic co-operation and development (OECD) guideline number 425. The limit test was performed initially. Swiss albino mice weighing 20–25 g were used in the toxicity study. Six mice were serially administered a 2000 mg/kg dose of extract prepared in water as recommended in the guideline. After dose administration, each animal was observed after every hour for signs of toxicity and abnormality in behavior up to the 48th hour. After this, daily observations for toxicity and mortality were made up to the 14th day. The body weights of the animals were recorded every third day. On the 14th day after dosing, all the mice were sacrificed and processed for gross necropsy.

2.7. Assessment of sedative activity

2.7.1. Measurement of locomotor activity

The locomotor activity was performed as per Adnaik et al. (2009), through actophotometer. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. Each mouse was placed individually in the actophotometer for 10 min and the basal activity score was obtained. Subsequently, the animals were divided into four groups, each consisting of six animals. First group received normal saline, *p.o.* Second group received standard drug diazepam 4 mg/kg, *i.p.* Third and fourth groups were administered AELVA (50 and 150 mg/kg, *p.o.*). After 30 min of *i.p.* and 60 min of *p.o.* dose respectively the mice were placed again in the actophotometer for observing the activity (Adnaik et al., 2009).