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Research Article

EVALUATION OF ANXIOLYTIC ACTIVITY OF METHANOLIC AND AQUEOUS ROOT EXTRACTS OF *COCCULUS HIRSUTUS*

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ABSTRACT

Cocculus hirsutus (L.) (Menispermaceae) is growing abundantly in different parts of India. *C.hirsutus* has been traditionally used for wide range of ailments, including constipation and kidney problems. The extracts of flowers, seeds, leaves and barks of *C. hirsutus* have been extensively studied for many potential uses including the anti-inflammatory and analgesic activities. *C.hirsutus* was subjected to preliminary anti-anxiety screening studies, with a view to ascertain the verity of its traditional use as an anxiolytic. In the present investigation, roots of the plant were extracted using solvents methanol and distilled water. All the crude extracts were evaluated for anti-anxiety activity in mice using light and dark transition model. Among all these extracts, only methanol extract exhibited significant anti-anxiety activity at a dose of 100 mg/kg and 200mg/kg in mice with respect to control as well as standard (diazepam, 2 mg/kg). In light dark transition model, diazepam-treated rats significantly increased the time spent in light zone and decreased the duration of immobility, while methanolic extract treated mice also showed a significant ($p<0.01$) increase in the time spent in light zone. Diazepam and the *C.hirsutus* extracts do not produced any overt motor dysfunction. These results indicate that *C.hirsutus* is an effective anxiolytic agent and could be useful in alternative treatment.

Keywords: Anxiety, *Cocculus hirsutus,* Diazepam, Motor dysfunction

INTRODUCTION

In present era, a sudden holocaust of mental disorders, and recognition of severe side effects and addiction liabilities associated with long term administration of widely prescribed synthetic drugs have aroused the attention of researchers towards natural resources. According to the World Health report (WHO, 2001), approximately 450 million people suffer from a mental or behavioural disorder, yet only a small minority of them receive even the most basic treatment. Anxiety disorder is a blanket term covering several different forms of a type of mental illness of abnormal and pathological fear and anxiety. It creates a condition of unexplained anticipatory fear and apprehension regarding the occurrence of even normal things in life[1]. Anxiety disorders only came under the aegis of psychiatry at the end of the 19th century[2]. Anxiety is a symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. It is a generalized mood condition that can often occur without an identifiable triggering stimulus [3, 4]. The light/dark box test measured the anxiety-related behaviour in mice.

Cocculus hirsutus (L.) Diels locally called Jaljamini, belonging to the family *Menispermaceae* is a climbing scandent shrub with hairy sepals[5]. Earlier investigation on the plant resulted in the isolation of several bioactive alkaloids and triterpenoids[6,7,8]. The methanolic extract of roots of C. hirsutus has a promising anti-arthritic activity.[9] *C. hirsutus* used medicinally by the Indian tribes for a wide range of ailments, including constipation and kidney problems[10]. The extracts of flowers, seeds, leaves and barks of *C. hirsutus* have been extensively studied for many potential uses including the anti-inflammatory and analgesic activities[11]. The objective of the present study was to evaluate the anxiolytic effect produced by the methanol and aqueous extract from *C.hirsutus* in mice.

MATERIALS AND METHODS

Plant material

The roots of *C.hirsutus* were collected from the forests of Pavagadh, Gujarat, India and authenticated at the department of bioscience, Vallabh vidyanagar, Gujarat. The roots were air-dried separately for 1 month and the respective material was powdered.

Drugs and Chemicals

Methanol LR grade were employed for the extraction of the plant material. Diazepam ampoule was procured from Sigma, India and was used as standard drugs. Two different concentrations (100 and 200 mg/kg) of the *C.hirsutus* extracts were prepared by dissolving the extracts in control (distilled water).

Preparation of Extract

The petroleum ether extract of root powder was prepared using petroleum ether (40-60^{o}C) by soxhlet method at a temperature of 40-60^{o}C. The methanolic extract was prepared using methanol by soxhlet method[12]**.** Aqueous extract was also prepared .The extracts were concentrated under vacuum and dried over anhydrous sodium sulphate. The methanolic extract yielded semisolid, viscous, dark coloured mass while aqueous extract yielded dark brown coloured mass.

Pharmacological screening for anxiolytic activity Animals

Swiss albino mice between 6 and 12 weeks old and weighing 22–34 g were procured. Mice were housed in cages of six at 22±2°C in a 12 h light/dark cycle. The mice were allowed standard food pellets and tap water ad libitum. Animals were habituated to laboratory environment for 48 h prior to experimental steps to minimize non-specific stress. Groups of five mice were used in entire sets of experiments. The Institutional Animal Ethical Committee approved the protocol of this study.

Acute Toxicity Studies

Methanolic extract of *C.hirsutus* at different doses (50-2000 mg/kg) was administered orally to mice. During the 24 hours after the drug administration, the animals were observed for gross behavioral changes such as hyperactivity, grooming, convulsions, sedation, hypothermia, and mortality were observed and doses selected were 100 mg/kg and 200 mg/kg, body weight.

Assessment of Anxiolytic Activity

The anxiolytic activity of *C.hirsutus* was examined using the Light dark transition model in mice. The animals were divided into six groups, consisting of six mice per group. A negative control group was included which received physiological saline solution (p.o.). Positive control groups were administered with diazepam 2 mg/kg; Groups 3 and 4 received C.hirsutus methanolic extract in a dose of 100 and 200 mg/kg, respectively; Groups 5 and 6 received C.hirsutus aqueous extract in a dose of 100 and 200 mg/kg, respectively; All treatments were administered 1 h before the test.

Light dark transition model

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. Each animal was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box [13,14]. The apparatus was cleaned thoroughly between trials. Diazepam dose of 2 mg/kg, i.p. was used as a standard compound.

Statistical analysis

All data are given as mean ± SEM and analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's test. The groups treated with *C.hirsutus* extracts were compared with the respective vehicle group. The diazepam-treated group was compared with vehicle. P values <0.05 were considered statistically significant.

RESULTS

Acute toxicity studies indicate that *C.hirsutus* root extract can be used safely in the animals up to a dose of 2000 mg/kg body weight. In general behaviour studies a slight reduction in the locomotor activity and grip strength was recorded. In this test the numbers of transitions between the dark and light compartments as well as the times spent in the light side are recognized as anxiety indices. Diazepam-treated rats significantly ($p<0.05$) increased the time spent in light zone and decreased the duration of immobility. *C.hirsutus* aqueous extract treated animals also showed a significant ($p<0.05$) increase in the time spent (100 and 200 mg/kg) in light zone.

C.hirsutus extracts reduced the duration of immobility at the highest dose (200 mg/kg of methanolic extract). An increase in the number of entries into light arena was significant. The mean time spent by the mice in open arms after oral administration of various extracts of *C.hirsutus* roots, diazepam (2 mg/kg) and the control (vehicle) has been shown in Table 1. Among the extracts tested, maximum anxiolytic activity was observed in the methanol at the dose of 200 mg/kg, p.o.

Table 1: Anti-anxiety effects of various extracts of *C.hirsutus*

Treatment	Dose	Average time spent in Open arms Mean ± S.D.	Number of entries in open arms Meann ± S.D.
Control	vehicle	6.900 ± 0.67	16.78±0.71
Standard	2mg/kg	14.02± 1.23	40.77±1.38
Methanol extract	100mg/kg	10.30±0.78 [a]	25.99±0.55 [a]
	200mg/kg	12.19±1.15[a]	24.59±0.69 [a]
Aqueous extract	100mg/kg	6.46±0.60[b]	14.36±0.20 [b]
	200mg/kg	6.072±0.91 [b]	12.57±0.50 [b]

Values are expressed by mean ± SD of six animals in each group

Statistical significance: a significant at $p<0.05$, b significant at $p<0.01$

DISCUSSION

The assessment of anxiety related behavior in animal model is based on the assumption that anxiety in animals is comparable to anxiety in humans. There has been a considerable popular interest in the use of herbal products, to treat anxiety reaction. It has been concluded that measurement of the time spent in the light zone, but not the number of transfers was the most reproducible and useful parameter for assessing anxiolytic activity[15]. The present data showed that *C.hirsutus* root extracts increase the time spent in the light zone, suggesting again these extracts possesses anxiolytic properties. Results of this study indicated that the methanolic root extracts of *C.hirsutus* had central anxiolytic effects. Data here obtained allows us to propose this plant species as an excellent candidate for isolating new substances with potential anxiolytic activity.

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