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Original Research Article

Behavioral effects of high altitude medicinal plant in rats

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Abstract: Anxiety disorders have consistently been found to be associated with substantial impairments in both productive and social roles. They are considered among the most prevalent psychiatric conditions, affecting the general population of industrialized societies. Since anxiety and panic disorders are the most common psychiatric disorders, one of the therapeutic approaches used is pharmacological intervention. Benzodiazepines have been used but there are clinical problems associated such as tolerance and addiction etc. Procedures ranging from yoga and meditation to anxiolytic drugs have been used to counter the aversive effects of anxiety, but appear to have limited use. A number of herbal medicines are commonly used for the treatment of neurological and psychological disorders. The present study aims to evaluate anxiolytic / anti-anxiety potential of the *Angelica glauca* in experimental model of anxiety. *Angelica glauca* (Gandhrayan) belongs to family Apiaceae. It is a high altitude medicinal plant of Himalaya. Species of *Angelica* have been used in ancient traditional medicine systems. But the plant has not been studied for its anxiolytic activity, which is likely to be present in view of its use to treat a wide variety of conditions. Methanolic extract of *Angelica glauca* was studied at graded doses to evaluate its anxiolytic effect. The anxiolytic activity was measured by behavioural observations conducted through elevated plus maze, open field and hole board test and compared with control and standard control. Result indicates that *Angelica glauca* possesses anxiolytic property.
Keywords: *Angelica glauca*, Anxiolytic, behavior.

INTRODUCTION

Anxiety disorders are the most prevalent conditions that affect emotion and cognitive behavior and is considered as one of the most common psychiatric disorders [1,2,3,4,5] which decreases the quality of life worldwide. Among therapeutic regimens, benzodiazepines and serotonin modulators are being extensively used to treat anxiety [6]. Benzodiazepines are among most frequently prescribed anxiolytics, but several clinical problems are associated with benzodiazepines viz. fairly high risk of dependence, tolerance and addiction in long term use [7,8,9,10] and gives adverse effects on behaviour, cognition, immunity, muscle relaxation etc [11,12]. Anxiolytics or cognitive behavioral therapy, yoga and meditation have been in practice [13, 14] but many patients remain untreated, experience adverse effects of drugs [15], or do not get benefited [16]. Till date efficacy of available drugs are limited. In such situation herbal medication may be considered as an alternative to complementary medicine. Use of medicinal plants as a therapeutic approach for psychiatric illness has increased significantly. A number of herbal medicines are being used for the treatment of neurological and psychological disorders [17].

Angelica glauca finds extensive use in traditional Indian medicine for the treatment of several diseases but has not been studied for its anxiolytic effect. *Angelica glauca* is native to India [8], which is distributed at altitudes of 2000-4000 meters world-wide [18]. In India, it is found sparsely distributed in North-West Himalayas [19]. *Angelica glauca* is a glabrous, aromatic herb 1.2-3.6 meters in height, roots are tuberous (20-50 cm. thick and spongy), belongs to family *Apiaceae* [18]. *Angelica* has recently become a very popular herb in the United States, and is often recommended by herbalists as a treatment for flatulence and stomach pains and as a stimulant to invigorate circulation and warm the body. Because of the diverse medicinal properties, several species from this genus have been investigated and used in various ailments globally. For centuries, many species of this genus, e.g. *Angelica acutiloba*, *Angelica archangelica*, *Angelica*

atropupurea, *Angelica dahurica*, *Angelica japonica*, *Angelica glauca*, *Angelica gigas*, *Angelica koreana*, *Angelica sinensis*, *Angelica sylvestris* etc, have been used traditionally as anti-inflammatory, diuretic, expectorant and diaphoretic and as remedy for colds, flu, influenza, hepatitis, arthritis, indigestion, coughs, chronic bronchitis, pleurisy, typhoid, headaches, wind, fever, colic, travel sickness, rheumatism, bacterial and fungal infections and diseases of the urinary organs [20]. The root is considered as cardio active and stimulant; carminative, expectorant, diaphoretic; useful in stomach ailments in adults and children [21] and also used in rheumatism and urinary disorders. The powdered root along with milk is used to treat bronchitis [20, 22]. *Angelica* contains various compounds such as; coumarins, acetylenic compounds, chalcones, sesquiterpenes and polysaccharides which may have potential medicinal properties [20]. Since anxiolytic properties of *Angelica glauca* have not been studied therefore the study is conducted for systematic pharmacological evaluation of anxiolytic activity in rats.

MATERIALS AND METHODS

Collection of plant material and preparation of standardized extract

Roots of *Angelica glauca* were collected from high altitude of Munsyari Tehsil in Pithoragarh district of Uttarakhand and were identified from the Department of Botany, Government Post Graduate College, Pithoragarh. Uttarakhand, India. Roots were used for methanolic extraction by percolation. The extracts of *Angelica glauca* prepared was used in the study. The standard drug used in the study, diazepam was purchased from Ranbaxy, India.

Animals

Naïve adult male Sprague-Dawley rats were housed in a departmental animal house registered under CPCSEA (Reg. No. 1449/GO/q/11/CPCSEA) in a group of 4 in polypropylene cages (38 X 23 X 10 cm) under standard housing conditions (temp., 24 ± 2°C; humidity, 60-65%) with 12-h light and dark cycle. Food was provided as dry pellets and water was available *ad libitum*. Rats were kept for 7 days in laboratory for habituation before experimentation. Experiments were conducted in accordance with our institutional laboratory animal ethical guidelines.

Experiment procedure

The animals were divided in five groups with six animals in each group and treatment was given according to the following schedule: Group 1 consisted of saline-treated rats which served as control, group-II consisted of animals treated with standard drug diazepam (0.25, 0.5, 1.0 mg/kg, p.o) and was considered as positive control and group III, IV and V were administered with crude methanolic extract of test drug (*Angelica glauca*) at the dose of (100, 200 and 400 mg/kg, p.o) respectively. All the drugs were prepared immediately before use and were administered orally. Experiments were conducted 30 minutes after vehicle/test/standard drug administration to the respective group.

A number of tests were required to establish the anxiolytic property of a drug andwidely accepted animal models were used in the study.

Elevated plus maze (EPM) [23, 24]

EPM is a reliable and pharmacologically sensitive paradigm based on the conflict between innate rodent desire to explore and the fear of open and elevated areas. The EPM consist of two open arms and two enclosed arms with open roof arranged such that the two open arms are opposite to each other. The maze is elevated to a height of 50 cm above the floor. To reduce stress, rats were handled by the investigator on alternate days. Thirty minute after the administration of test drug *Angelica glauca* (100, 200 and 400 mg/kg, p.o); standard drug diazepam (0.25, 0.5, 1.0 mg/kg, p.o) and vehicle, one rat in one time was placed in the center of the maze. During 5 min test observation period, the time spent in both open and closed arms was recorded. The number of entries in closed and open arms (all four paws in open arm) was counted.

Open Field Test [25, 26]

The open field test is simple and the most frequently used model to study anxiety in rats. The apparatus consists of a wooden box (60x60x30 cm).The entire apparatus was painted white except for 6 mm thick black lines that divided the floor into 16 squares. The apparatus was illuminated with 150-200 lux in the centre of the open field arena. After 30 minutes of test drug *Angelica glauca* (100, 200 and 400 mg/kg, p.o), standard drug diazepam (0.25, 0.5, 1.0 mg/kg, p.o) and vehicle treatment, rats were placed singly in central position and allowed to explore the apparatus freely.During 5 min of experiment different behavioral parameters like number of square crossed, time spent in centre, time spent in perimeter, rearing and assisted rearing were noted.

Hole-board test [25, 27]

The hole-board apparatus is made of a rectangular wooden box (60×60×35 cm) with equidistant holes of 2 cm diameter in the floor. The floor of the box is kept 12 cm above the ground. Rats were treated with test drug *Angelica glauca* (100, 200 and 400 mg/kg, p.o), vehicle and standard drug diazepam (0.25, 0.5, 1.0 mg/kg, p.o), and after 30 min the rats were placed singly in the center of the hole-board, and during a 5-min trial the number of head dips, the time spent in head-dipping, number of rearing, and number of assisted rearing were recorded. A head dip scores if both eyes disappear into the hole [28].

Apparatuses were cleaned thoroughly before and after each trial to remove any trace of odor. Test

sessions were recorded via an overhead video camera linked to a monitor for record and future analysis. All behavioral recordings were carried out with the observer unaware of the treatment of the rat.

Statistical Analyses

The statistical analysis of the data was done using one way analysis of variance. All the data were presented as mean ±SEM values. A probability of less than 0.05 was considered statistically significant.

RESULTS

Elevated Plus Maze (EPM) Experiment

Elevated plus Maze results are summarized in table-1. It shows that parameters; time spent in open arms and numbers of entries in open arms were increased and time spent in closed arms was decreased as compared to control and at all doses of *Angelica glauca*. *Angelica glauca* at the dose of 200 mg/kg significantly altered all parameters; whereas 100 mg/kg dose produced enhanced time spent in open arms and decreased time spent in closed arms markedly.

Table-1: Result of anxiolytic activity of *Angelica glauca* on Elevated Plus Maze Test

Drug	mg/kg po n=6	Number of entries in open arms	Time spent in open arms	Number of entries in closed arms	Time spent in closed arms
Control		2.1±0.75	13.5±1.87	3±0.89	232±10.02
Angelica glauca	100	3.00±0.95	30.67±4.22*	3.33±1.16	189.00±13.73*
	200	4.17±1.07*	32.33±3.98*	5.50±1.43*	167.00±11.02*
	400	2.17±0.73	19.00±3.76	3.33±1.03	226.50±14.43
Diazepam	0.25	3.6±0.82	24.5±1.76	2±0.52	154±7.63
	0.50	3.8±0.75	27.16±3.60	2.1±0.75	143.5±7.56
	1.00	4±0.89	26.5±3.27	2.5±0.55	153.3±5.96

Values represent the group mean± SEM, (n=6), *p<0. 05 vs. control

Hole-Board test (fig.1)

Number of head dips, time spent in head dipping and number of assisted rearing were increased markedly at all doses of *Angelica glauca* but no significant changes were observed in number of rearing at all doses of *Angelica glauca* as compared to control.

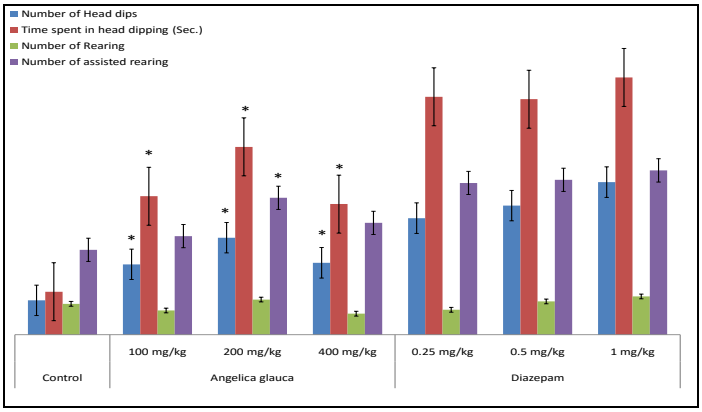

Fig-1: Effect of *Angelica glauca* in Hole-Board test. Values represent the group mean± SEM, (n=6), *p<0. 05 vs. control

Open Field Experiment (Fig-2 and Fig-3)

Extract of *Angelica glauca* at the dose of 100 and 200 and 400 mg/kg, p.o produced marked increase in time spent in the centre and assisted rearing as compared to control. Whereas, number of squares crossed and rearing was mildly increased while time spent in perimeter was decreased significantly at all doses of *Angelica glauca* as compare to control.

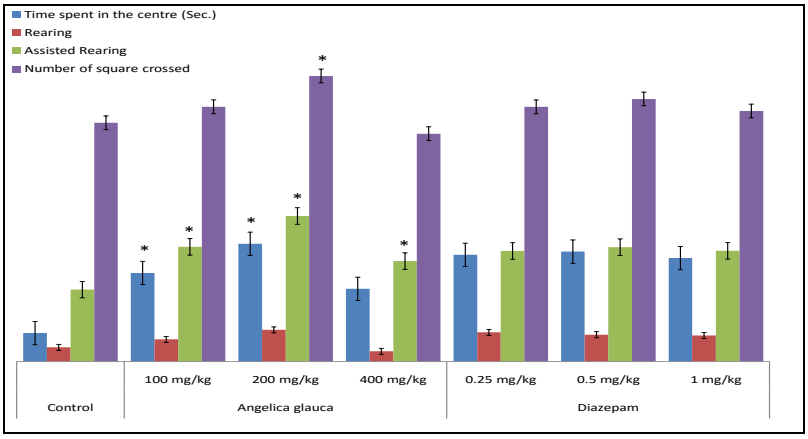

Fig-2: Effect of *Angelica glauca* in Open Field test. Values represent the group mean± SEM, (n=6), *p<0. 05 vs. control

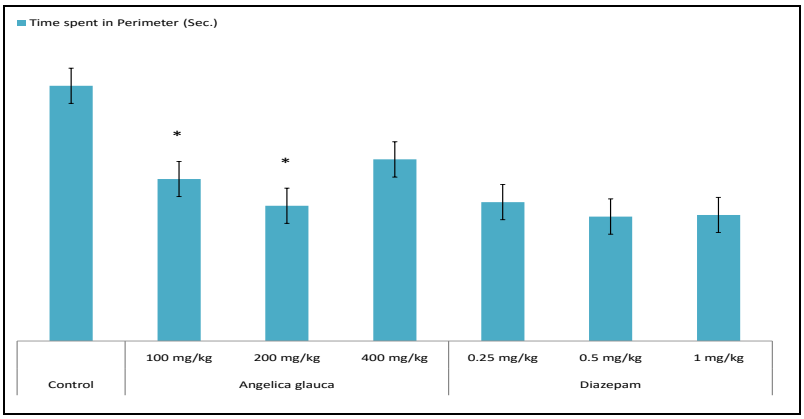

Fig-3: Effect of *Angelica glauca* for time spent in the perimeter (Sec.) in Open Field test. Values represent the group mean± SEM, (n=6), *p<0. 05 vs. control

DISCUSSION

Prolonged stressful conditions have been associated with dysfunction of several neurotransmitters [30] resulting in behavioral changes as well as a cascade of hormonal release from the hypothalamus–pituitary–adrenal (HPA) axis leading to disorders like anxiety and depression [31, 32]. Anxiety has been postulated to be involved in the etiopathogenesis of psychosomatic disorders including psychiatric disorders such as psychoses and depression; immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer; hypertension and ulcerative colitis. The failure of successful adaptation during stressful situations may lead to illnesses that result from or are associated with dysregulation of the stress response [29] and results in anxiety disorders. A number of herbal medicines are commonly used for the treatment of neurological and psychological disorders [33]. It is evidenced that secondary metabolites of several plants used in the treatment of psychiatric disorders especially for anxiety in traditional system of medicine, directly or indirectly facilitates the effect of CNS, neurotransmitters especially noradrenalin, γ-aminobutyric acid (GABA), dopamine and 5-hydroxytryptamine activities [34, 35, 36, 37, 38]. Mechanism of action of anxiolytic plants may have interaction with some of the natural endogenous mediators in the body as reported by several scientific communities [41]. It is evident that there could be a linkage in the interaction of serotonergic pathways and plant extract [42, 43]. 5HT subtype, $5HT_{1A}$ has been considered the main serotonin receptor implicated in fear and anxiety and $5HT_{1A}$ receptor partial or total agonist showed anxiolytic properties [44].

Elevated plus maze test is most widely used behavioral test in rats for screening putative anxiolytics. The decrease in the aversion to the open arms is the result of an anxiolytic effect [39]. Pretreatment with methanolic extract of *Angelica glauca* roots increased the time spent in open arms and decreased time spent in closed arms significantly at 100 and 200 mg/kg doses. Number of entries in open arms and closed arms were also increased in all three doses but no significant changes were observed at 400 mg /kg dose as compared to control group. The Hole-Board model was used to analyze head dipping behavior which is sensitive to changes in the emotional state of the animal and indicates that the expression of an anxiolytic treated animals may be reflected by the enhanced behavior of head dipping [40]. Number of head dips, time spent in head dipping and number of assisted rearing was increased markedly at all three doses of *Angelica*

glauca as compared to control animals. The Open field test showed prominent effect at the doses of 100 mg/kg and 200 mg/kg of *Angelica glauca* but 400 mg/kg dose showed mild decseases as compared to control animals. The gross behavior activity such as gait, ptosis, piloerrection, tremors, lacrimation, urination, writhing reflexes, pineal reflexes corneal reflexes and straub tail were found normal after treatment with crude extract of *Angelica glauca*.

CONCLUSIONS

The data reported has an evidence for the anxiolytic activity of the crude (methanolic) extract of *Angelica glauca*. The effect of *Angelica glauca* may affect certain endogenous mediators to reduce anxiety. Further study is needed to explore the mechanism of action of *Angelica glauca* for its antianxiety or anxiolytic activity.

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