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Research Article

Experimental studies on *Centella asiatica* for anxiolytic activity in rats

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Abstract: Anxiety disorders are considered as one of the most prevalent psychiatric syndromes. They are associated with substantial impairments in both productive and social roles. Several clinical problems are associated with the anxiolytics being prescribed and therefore herbal medicines are being considered as an alternative to the complementary medicine. In the present study methanolic extract of *Centella asiatica* at the dose of 100, 200 and 400 mg/kg, (p.o) in male Sprague-Dawley rats was studied for its anxiolytic property in widely accepted animals models viz. open field, elevated plus maze and hole board. The open field test marked increase in rearing, assisted rearing and number of square crossed and time spent in the center of arena. In the hole board test, enhanced time of head dipping and number of head dip in the treated animals was observed as compared to control. Similarly in elevated plus maze test, a marked increase in the number of entries and the time spent in open arms was noticed as compared to closed arms. Thus the results obtained indicate that *Centella asiatica* imparts potent anxiolytic activity.
Keywords: Anxiety, Behaviour, *Centella asiatica*, Diazepam

INTRODUCTION

Anxiety is one of the most common psychiatric disorders [1,2] which decreases the quality of life worldwide. It is considered among the most prevalent psychiatric syndromes affecting 10 to 30 % of the general population of industrialized societies [3,4] that affect emotion and cognition and also exhibit >50% co-morbidity with depression [5]. Benzodiazepines are among most frequently prescribed anxiolytics, but now it is widely accepted that several clinical problems are associated with benzodiazepines viz. fairly high risk of dependence, tolerance and addiction in long term use [6,7,8]. Abuse liability has also been documented among vulnerable groups [9] and adverse effects on behavior, cognition, immunity, muscle relaxation etc [10,11]. Anxiolytics or cognitive behavioral therapy has been in practice [12] but many patients remain untreated, experience adverse effects of drugs [13], or do not get benefited [14]. Till date efficacy of available drugs are limited. In such situation herbal medication may be considered as an alternative to complementary medicine. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy [15]. 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 [16].

Centella asiatica (L.) is a herbaceous creeping plant belonging to family *Apiaceae*. In Ayurveda it is known as Brabhnmi, in Unani medicine Madukparni and Gotu Kola in the western world. It has been used for centuries in Ayurvedic and traditional Chinese medicine to alleviate symptoms of depression and anxiety and helps in sleep disorders. The pharmacological studies on *Centella asiatica* have been reported by several research workers in *in vitro* and *in vivo* models. *Centella asiatica* enhances tranquillizing activity in animals [17], increases phenobarbitone induced sleeping time and decrease immobility in forced swimming test [18]. It is used in some CNS and gastrointestinal disorders [28], it improves learning and memory processes *in vivo* [19], improves general mental ability of mentally retarded children [20], improves maze learning in rats [21] and has beneficial effect on cognitive functions and oxidative stress in rats [22]. *Centella asiatica* prevents ethanol induced gastric mucosal lesions and reduces damaging effect of free radicals [23], shows healing effect on gastric ulcers in rats [24]. Anxiolytic activity of asiaticoides from *Centella asiatica* has been reported in mice [25] and in rats [26, 27]. Since, very few studies have been conducted on the anxiolytic activity of the *Centella asiatica*, therefore the present study has been conducted to evaluate the anxiolytic effect in the methanolic extract of whole plants of *Centella asiatica* in universally accepted murine models of anxiety.

MATERIALS AND METHODS

Collection of plant material and preparation of standardized extract:

Whole plants of *Centella asiatica* were collected during month of September-October from the campus of the college, Pithoragarh, Uttarakhand and were identified by the Department of Botany, LSM Govt. Post Graduate College, Pithoragarh. After collection the plants were washed with distilled water

and dried in the ventilated shed area in the lab. Air dried whole plants were crushed into 40 mess size for the extraction. The crushed plants were soaked in methanol for 48 hours, decanted, filtered through muslin cloth and Whatman's filter paper No. 1. The filtrate was concentrated by evaporating methanol by distillation process at 50-60^{0}C. The residue obtained after removing the solvent was transferred to a petri dish and kept over water bath at 40-50^0 C till the solvent was completely evaporated. After complete removal of solvent it was stored at 4^{0}C for future use.

The standard drug used in the study, Diazepam was purchased from Ranbaxy, India and methanolic extraction of *Centella asiatica* was done in the department.

***Animals and treatment schedule*:**

Male Sprague-Dawley rats (225-250 g) were housed in group of 6 animals in polyethylene cages (38 X 23 X 10 cm) under controlled conditions of temperature 22±2 °C, relative humidity 60±10%, and 12-h light-dark cycle. Food was provided in dry pellets and water was available *ad libitum*. The experiments were performed as per approval of the Institutional Animal Ethical Committee under the guidelines of CPCSEA, Govt. of India (Reg. No. 1449/GO/a/11/CPCSEA). Rats were kept for 7 days in laboratory for habituation before experimentation. The animals were divided in five groups with six animals in each group and treatment was given 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 (*Centella asiatica*) at the dose of (100, 200 and 400 mg/kg, p.o) respectively. The dose of *Centella asiatica* was determined on the basis of the initial pilot study. In pilot study, increasing doses of *Centella asiatica* (50, 100, and 200 and 400 mg/kg, p.o.) were administered in the normal and anxiety group of rats in order to evaluate their effect per se and to find out the effective dose for further use in experimental studies. All the drugs were prepared immediately before use and were administered PO in a volume of 10 ml/kg body weight. Experiments were conducted 30 minutes after vehicle/test/standard drug administration to the respective group.

Experiment procedure:

A number of tests are required to investigate the anxiolytic properties of a drug. In this study the animal models of anxiety included are widely accepted.

Elevated plus maze Test [29]

Elevated plus maze is one of the most widely used behavioral anxiety test in rats for screening putative anxiolytics. The EPM consists of two open arms of 50x10x40 cm, and two enclosed arms of 50X10X40 cm with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated to a height of 50 cm above the floor. The rats were housed in pairs 10 days prior to testing in the apparatus. To reduce stress, rats were handled by the investigator on alternate days. Thirty minute after the administration of test drug *Centella asiatica* (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.

Hole-board test [30]

The hole-board apparatus is made of a rectangular wooden box (60×60×35 cm) with four equidistant holes of 2 cm diameter in the floor. The floor of the box is kept 12 cm above the ground and divided into nine (20×20 cm) squares. Rats were treated with test drug *Centella asiatica* (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 [31].

Open Field Test [30, 32]

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 base of box is painted grey or black and is divided into 16 equal squares (15x15 cm). The apparatus was illuminated with 150-200 lux in the centre of the open field arena. After 30 minutes of test drug (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. In the 5 minutes session the behavioural end points recorded were: number of rearing (if possible), number of assisted rearing (forepaw touching the wall of the apparatus) number of squares crossed and time spent in the central and peripheral zone of the arena.

All the apparatus were cleaned thoroughly before and after each trial to remove any trace of odor. The experiments were conducted in a sound attenuated room and 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 Analysis:

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 0.01 was considered to be statistically significant.

RESULTS AND DISCUSSION

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 [10]. The failure of successful adaptation during stressful situations may lead to illnesses that result from, or are associated with dysregulation of the stress response [33] and results in anxiety disorders. Prolonged stressful conditions have been associated with dysfunction of several neurotransmitters [34] 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 [35, 36].

A number of herbal medicines are commonly used for the treatment of neurological and psychological disorders [37]. 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 [38, 39, 40, 41, 42]. *Centella asiatica* is a plant traditionally used in various ailments and very few studies have been conducted on its anxiolytic activity. Preliminary findings suggest that *Centella asiatica* has anxiolytic activity in humans [43] at the dose of 12 g. One of the most widely used behavioral test in rats for screening putative anxiolytics is elevated plus maze test. The EPM evokes conflict between the need to explore the novel area and need to avoid more vulnerable (or aversive) areas of the EPM (height and open space) [44]. The decrease in the aversion to the open arms is the result of an anxiolytic effect, expressed by the enhanced time spent and number of entries in open arms and can be increased by anxiogenic drug [45]. In our studies too, methanolic extract of *Centella asiatica* significantly increases the time spent in open arms and number of entries in open arm while time spent in closed arms decreased significantly indicating that the plant showed antianxiety activity (Table-1). The open field test showed that administration of *Centella asiatica* increased the time spent in the centre of the arena and increased rearing and assisted rearing and number of square crossed as compared with control animals. This observation supports the view that the crude extract imparts anxiolytic activity. No dose-dependent response was observed in the given dose range Table-2. The hole board model is 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 [46]. *Centella asiatica* at the doses of 100, 200 and 400 mg/kg, (p.o) increased the number of head dips, time spent in head dipping compared with the control. The number of rearing and assisted rearing was not affected significantly Table-3. 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 *Centella asiatica*.

Table-1: Result of anxiolytic activity of methanolic extract of *Centella asiatica* on Elevated Plus Maze Test

	mg/kg p.o. (n=6)	**Number of entries in open arms**	**Time spent in open arms**	**Number of entries in closed arms**	**Time spent in closed arms**
Vehicle	vehicle	2.1±0.75	13.5±1.87	3±0.89	232±10.02
Methanolic Extract	100	3.16±0.75	26.33±2.25*	3.83±0.75	183.16±9.83*
	200	4±0.89*	27.5±1.97*	3±0.63	184.1±6.85*
	400	4.5±1.05*	13.33±1.75	3.1±0.75	194.5±5.17
Diazepam	0.25	3.6±0.82	24.5±1.76	2±0.52	154±7.63
	0.5	3.8±0.75	27.16±3.60	2.1±0.75	143.5±7.56
	1.0	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

Table-2: Result of anxiolytic activity of methanolic extract of Centella asiatica on Open field experiment

	mg/kg p.o. (n=6)	Time spent in the centre	Time spent in Perimeter	Rearing	Assisted Rearing	Number of square crossed
Vehicle	vehicle	6.±1.16	277.8±9.6	3±0.89	15.16±2.40	50.3±4.16
Methanolic Extract	100	8.66±1.03	268±15.9	3.66±0.81	23.16±3.31*	55±2.89
	200	10±1.54*	258.83±9.98	5.3±0.81*	23.6±2.4*	58±2.60
	400	8.3±1.75	251.83±9.78	6±1.4*	25±4.93	50.8±5.26
Diazepam	0.25	22.5±1.37	256±4.41	6.16±1.16	23.3±1.96	53.66±3.44
	0.5	23.16±1.47	253.3±3.44	5.66±0.81	24.1±2.31	55.33±4.5
	1.0	21.8±1.47	253.6±4.32	5.5±1.04	23.33±1.96	52.8±4.26

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

Table-3: Result of anxiolytic activity of methanolic extract of *Centella asiatica* on Hole-Board Test

	mg/kg p.o. (n=6)	Number of Head dips	Time spent in head dipping	Number of Rearing	No. of assisted rearing
Vehicle	vehicle	7.33±0.81	9.16±1.47	6.6±0.81	18.1±1.94
Methanolic Extract	100	18±1.78*	38.1±3.8*	5.33±0.81	21.16±1.76
	200	17.16±1.16*	41±3.46*	5.8±1.47	23.16±2.74
	400	18.16±1.21*	40.16±2.92*	5±0.89	25.83±3.60
Diazepam	0.25	24.83±1.72	50.66±4.41	5.33±0.81	32.33±2.94
	0.5	27.5±1.87	50.16±3.60	7.1±1.47	33±2.82
	1.0	32.5±1.51	54.8±4.45	8.16±0.98	35±2.09

Values represent the group mean± SEM, (n=6), *P<0.05 vs. control

No biological cause has been identified for anxiety disorders. Heisler (1998)[47] suggested that 5HT subtype, $5HT_{1A}$ has been the main serotonin receptor implicated in fear and anxiety and $5HT_{1A}$ receptor partial or total agonist showed anxiolytic properties. According to McEwen (2000) HPA axis dysregulation caused by stress results in excess production of noradrenalin and corticosterone, sensitizes peripheral inflammatory response [48], and increases anxiety [49]. Engelmann (2004) [50] showed that repetitive stress exposure leads to enhanced release of corticotrophin releasing hormone (CRH). De Souza (1995) [51] has shown that CRH acts as neurotransmitter or neuromodulator and is implicated in the control of anxiety too [52]. Stress hormones (corticosterone) also affect bio-availability of neurotransmitters [53] and metabolic processes [54], thus affecting normal functioning of psychological and physiological processes.

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 [55, 56]. It is evident that there could be a linkage in the interaction of serotonergic pathways and plant extract [57, 58]. 5HT subtype, $5HT_{1A}$ has been considered the main serotonin receptor implicated in fear and anxiety and $5HT1_A$ receptor partial or total agonist showed anxiolytic properties [47]. Breier and Paul, (1990) [8] indicated that benzodiazepines / γ-aminobutyric acid (BZ/GABA) receptor complex is involved in the pathogenesis of anxiety and benzodiazepines produce their effects by facilitating GABA neurotransmission[59]. Thus the result of the present investigation indicates that *Centella asiatica* possessed potent antianxiety activity that was found in all the behavioral model of anxiety.

Conclusions

This study used several animal models of anxiety and thus provides support to the ayurvedic claim that *Centella asiatica* has anxiolytic activity. The data reported herein has an evidence for the anxiolytic

activity of the crude plant material and may affect certain mediators to reduce anxiety.

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