



ISSN (E): 2277- 7695  
 ISSN (P): 2349-8242  
 NAAS Rating: 5.03  
 TPI 2019; 8(8): 330-335  
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 www.thepharmajournal.com  
 Received: 10-06-2019  
 Accepted: 12-07-2019

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## Anxiolytic assessment of *Centella asiatica* with elevated zero maze and elevated plus maze in rats

**Sanjesh Kumar and Mansi Singh**

### Abstract

**Objective:** In this study, we evaluated the pharmacological effect of the *Centella asiatica* in the anxiety disorders with the help of the help of Elevated zero maze and Elevated plus maze apparatus in rats. The present study focuses on the Pharmacological evaluation of the *Centella asiatica* for anxiolytic activity with its ethanolic extract with the help of the Elevated zero maze and Elevated Plus maze apparatus in rats. For the study, the rats were divided into 4 groups and named or tagged properly. Group-I consisted of the rats that were administered Normal saline (2ml/kg) which served as control group, Group-II consisted of rats that are treated with the standard drug Diazepam (1.0mg/kg) and both the groups were treated 30min. prior to the experiment. *Centella asiatica* extract was administered at doses of Group-III 200mg/kg (T1) and Group-IV 400mg/kg (T2) body weight. The treated, standard groups are compared with control group and values are represented as the mean $\pm$  SEM; n=3;  $P<0.05$ ;  $P<0.01$  vs control. The following study found that there was enormous decline in the anxiety levels of the subjects and even the drug helps to replenish the decreased neurotransmitter levels in the brain.

**Keywords:** Anxiety, *Centella asiatica*, behavior, diazepam

### Introduction

Anxiety refers to the intrinsic, adaptive mechanism that tends the body to respond to existent or illusory dangers and problems. However, in case anxiety becomes pathological it shows severe emotional and physical consequences [1]. Anxiety is defined as an emotional state represented by a feeling of worry, apprehension, nervousness or discomfort concerning an imminent or anticipated event whose resolution is uncertain [2]. It is thought to be amongst the most widespread psychiatric syndromes touching 10 to 30% of the common population of world effecting feeling and cognition. It also demonstrates co-morbidity with depression [3]. The most well known symptoms include restlessness, insomnia, fatigue, headache, impaired concentration, confusion, irritability. Exposure to certain substances and drugs can trigger anxiety symptoms which include – caffeine, cannabis, cocaine and drugs like salbutamol, insulin, thyroid hormones etc. [4]. Benzodiazepines are the class of medications that are recommended to reduce the symptoms of anxiety. All benzodiazepines in clinical use have capacity to promote the binding of the major inhibitory neurotransmitter Gama-amino butyric acid (GABA) to the GABA  $\alpha$  sub-unit of GABA 330receptors, which exist as multi-subunit, ligand gated chloride channels, thereby enhancing the GABA –induced ionic currents through these channels [33]. But these present a vast array of abnormalities including insomnia, mania, violence and paranoia. The area of concern regarding the use of benzodiazepine in treatment of anxiety is the potential for habituation, dependence and abuse. To overcome these prevailing situations there is an immense need to switch over medications that overcome these predicaments. Herbal medications are alternatives with least adverse effects for anxiety disorder. So, the scientist favors utilization of Natural Plants with little side effects. Many plants are accounted to possess anxiolytic activity [5]. *Centella asiatica* (Apiaceae), commonly known as Gotu kola is a creeping plant. It is a slender ground creeping perennial herb, rooting at the nodes, and growing up to 2m long. Its leaves are glabrous, orbicular-reniform, and shallowly crenate. It is a native of India, China and South East Asia. In India it is the most widely exported and imported medicinal plant [6]. *Centella asiatica* is used as an –Antioxidant and Antibacterial Anti- inflammatory, Wound Healing, Learning and Memory, Anti Diabetic, Skin Aging and have Anxiolytic effect [7]. *Centella asiatica* embrace polyacetylenes, flavonoids, flavones, sterols, and lipids. The Plant even contain significant amount of amino acids, fatty acids, alkaloids, saccharides and inorganic salts [34]. *Centella asiatica* rendered many triterpenes namely Asiatic acid, madecassic acid, asiaticoside and madecassoside

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responsible for its medicinal properties [8]. *Centella asiatica* leaves are used to treat the anxiety disorders in India and other countries. The drug consists of fresh or dried whole herb with faint and characteristic aromatic odour with slight bitter taste [35]. The aim of this immediate study is to study the anxiolytic effect of *Centella asiatica* in rats. Since, in the present scenario of the synthetic drugs with huge amount of the adverse effects the present study on the herbal drug enlightens a new alternative for the treatment of the anxiety disorders without causing any harm to the patients.

**Materials and Methods**

**Collection of the plant material and preparation of the standardized extract**

The leaves of *Centella asiatica* was procured from the campus and was dried under shade. The plant leaves was identified by botanist at M.J.P. Rohilkhand University. A voucher specimen of the plant material was deposited at department of plant science, M.J.P Rohilkhand University, Bareilly. The fresh leaves were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were grounded into coarse powder by mechanical support (pulverizer). The dried leaf powder sample was extracted in Soxhlet apparatus at 60-70oc for 6 hours continuously in 70% ethanol. The extracted material was evaporated into dryness under reduced pressure at 40-50oc in vacuum rotary evaporator and stored in sterilized air tight container at 4oc for further use [14].

**Animal and treatment schedule**

Male rats six in each group having weight (150-200g) was used for the antianxiety activity. The animals were housed at temperature 22 ± 2%, relative humidity 60 ± 10% and 12h light and dark cycle. All the study was performed in Department of pharmacy M.J.P Rohilkhand University, Bareilly as per the guidelines of the Institutional Animal Ethical Committee (IAEC) and Committee for purpose of control and supervision of experiments on animals (CPCSEA). The rats were divided into 4 groups and named or tagged properly. Group-I consisted of the rats that were administered Normal saline (2ml/kg) which served as control group, Group-II consisted of rats that are treated with the standard drug Diazepam (1.0mg/kg) and both the groups were treated 30min. prior to the experiment. *Centella asiatica* extract was administered at doses of Group-III 200mg/kg (T1) and Group-IV 400mg/kg (T2) body weight [15]

**Table 1:** Different treatment given to the groups of rats.

Groups	Treatment
Group- I	Normal Saline (0.9%w/v)
Group-II	Standard drug Diazepam (1.0mg/kg, p.o.)
Group-III	200mg/kg Body weight Drug Extract
Group-IV	400mg/kg Body weight Drug Extract

**Experimental procedure**

There is variety of the animal models available for the screening of the anxiolytic effect of the drugs. In this study, most accepted models are being considered for the study.

**Elevated zero maze**

The Elevated zero maze test is animal pharmacologically validated anxiety test based on the natural aversion of rodents (rats/mice).The Elevated plus maze comprises a straannular black Perspex platform (10.5cm in diameter,10cm wide)raised

to 65cm from the ground and divided into four quadrants. The two opposite quadrants are surrounded by a black Perspex wall (height 27cm)on the inner outer edge of the platform, while the remaining two opposite quadrants are surrounded by a Plexiglas’s border (height 1 cm) which serves as Animal touch guidance in these open areas [27]. The rats are placed in one of the closed quadrants during a test period of 5 minutes. The elevated plus maze is cleaned with a 10% ethanol / water solution and dried between test sessions. The time spent in the open quadrant, the number of head dips on the edges of the platform and the number of extended positions reached from the open arms to the open arms is recorded. For one test, the animal was placed in one of the open parts facing the closed part of the apparatus and was observed for 300s. The behavioral measures taken included: (a) latency, that is, the time of first entry with all four legs from the closed to the open; (b) number of open entries; c) time spent in the open area; d) the number of line crossings in the open parts; e) the number of head dips on the edge of the platform; (f) the number of assisted stretching postures [16].



**Fig 1:** Elevated zero maze

**Elevated Plus Maze**

Elevated plus maze (EPM) was used for the study; it is the most frequently used for testing behavioral anxiety in rats. The EPM consists of two open arms of 50×10×40 cm with an open roof, arranged so that the two open arms are opposite to each other. The maze is elevated at a height of 50cm. During the trial test the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: time spend by the rat in the open and the closed arm respectively. The time of permanence measures the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the animal’s aversion to the open arms and promote the exploration thereof [17].



**Fig 1:** Elevated plus maze

## Behavior Coding

An animal was judged for the period of 5 minutes for the behavioral parameters on both the maze apparatuses. The total times spend by the rat on the open arm and the closed arm was recorded on the Elevated plus maze and Elevated zero maze apparatus. The rat was placed on the central square of the Elevated plus maze facing towards the open arm and closed quadrant of the Elevated zero maze apparatus [23]. Open arm entry- Frequency with which the animal entered the open arms. All the four paws of the rat should be on the open arm to be counted as an entry. Closed arm entry- Frequency with which the animal entered the closed arms. All the four paws of the rat should be on the closed arm to be counted as an entry [24].

## Results

*Centella asiatica* is investigated for the antianxiety activity in rats. The elevated plus maze and elevated zero maze evokes the conflict between the need to explore the novel area and need to avoid more vulnerable area<sup>1</sup>. Anxiety refers to the state in which fear seems to arise from an unknown source. About 10-30% of the common world population is affected by the psychiatric syndrome. The psychiatric disorder majorly affects cognition and feelings [3]. Exposure to certain substances like caffeine, cocaine, cannabis and drugs like salbutamol, thyroid hormones also induces anxiety<sup>4</sup>. The state affects the daily to daily life at broad scale and hinders individual thinking.

Treatment of the anxiety disorders with various medicines and therapies is not optimal till date and requires development of more effective newer drugs. Most widely prescribed drugs for the treatment of the anxiety disorder is Benzodiazepines. The use of these drugs is progressively declined due to the abuse liability, addiction, and associated adverse effects [28]. The common adverse effects of these drugs are insomnia, mania, violence and paranoia so; it's time to switch over to the medications that are well tolerated with maximum effectiveness and present least amount of the side effects [7]. In the present study *Centella asiatica* is investigated for its antianxiety activity. It is also used in the treatment of the cognitive disorders, wound healing, depression, diabetes, skin aging and inflammation [5]. To evaluate the antianxiety activity various animal models are available. Therefore, elevated plus maze and elevated zero maze is selected for the study. They are simple to operate, less time consuming and presents good results. The two mazes are used to evaluate the behavioral patterns in rats. The Elevated plus maze consists of the two open arms and two closed arms with the roof open arranged in a way so that two open arms are opposite to each other. The maze is elevated to a height of the 50cm. and behavioral trial of 5min was conducted on the maze for the evaluation [25]. On the other hand elevated zero maze is also similar but consists of the circular arena and elevated at a height of the 65cm with two open arms and two closed arms [26]. The animal is placed in the center facing one of the open arms and time spend by the rat in the open arm and closed arms is noted for a period of 5min to study the behavior elicited [18]. The study was conducted for the 7 days. On the basis of the evaluation methods following results are obtained from various groups of rats by Elevated plus maze and Elevated zero maze apparatus.

Control group consists of six animals was treated with normal saline (2ml/kg,p.o.) and *Centella asiatica* extract group was treated with extract at doses of 200mg/kg and 400mg/kg,p.o.

30 min. prior to the anxiety experiment. Control group animals spend more time in the closed arms of the elevated plus maze and elevated zero mazes. Whereas Drug extract group animals spends more time in the open arms of the mazes as compared to the control group, this is may be due to the activity of neurotransmitter GABA is enhanced by the *Centella asiatica* that resolves the conflict of avoiding open spaces by animals [19].

Diazepam (standard drug) group was given dose of 1.0mg/kg, p.o. and *Centella asiatica* extract groups at doses 200mg/kg, p.o. and 400mg/kg, p.o. consisted of the six animals each and the animals were treated with the drugs 30min prior to the experiment<sup>29</sup>. When both the groups were compared than it was noted that animals of the both the groups explored the open arms of the both mazes and spends more time in the open arms and the behavior shown by the rats proved that anxiety is being diminished to a great extent [20].

*Centella asiatica* extract groups treated at 200mg/kg and 400mg/kg, p.o. when compared at the mazes showed that the animals which were treated with doses 400mg/kg spend more time in the open arms as compared to 200mg/kg group. The animal tries to explore the open elevated areas of the mazes and this shows decline in their behavior of fear and anxiety. The animals were administered freshly prepared drug extract 30min before the anxiety experiment [21].

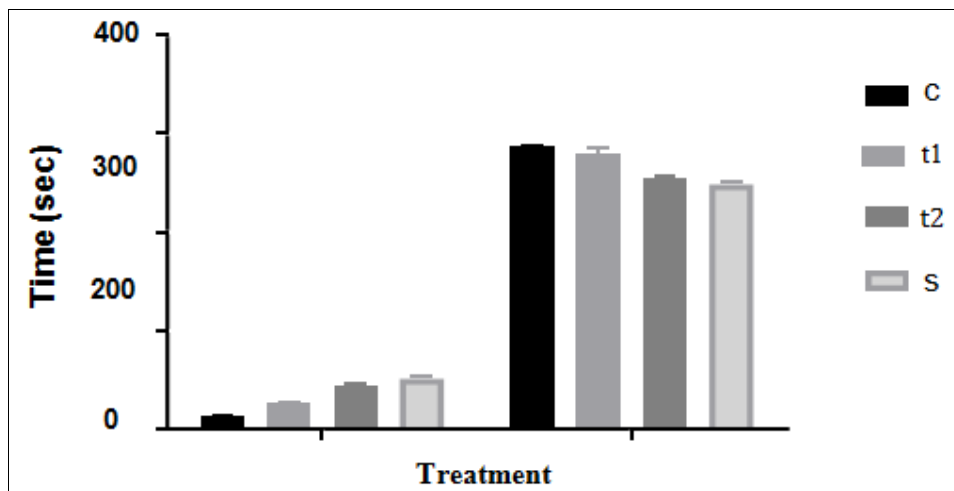
Control group, Diazepam group(standard drug) and *Centella asiatica* extract groups when compared for their effects on the behaviors exhibited by the rats on two mazes shows that the herbal drug *Centella asiatica* proved to significantly effective in relieving rats from the anxiety provoked by the elevated plus maze and elevated zero maze apparatuses simultaneously. *Centella asiatica* shows this effect may be due to their GABA modulation activity and thus shows agonist activity on the GABAB receptor [30]. The overall neurotransmission is also enhanced by the drug [22]. The rats of the herbal extract groups spend more time in the open arms and shows antianxiety activity. The decrease in aversion to the open arms is the result of the antianxiety effect of *Centella asiatica*. The study proved that the *Centella asiatica* posses the antianxiety properties.

The groups are evaluated using the Elevated Plus Maze and Elevated Zero Maze apparatus, respectively. Each group consists of 6 rats each and the Treatment schedule for each group is as follows: - Group-I consisted of the rats that were administered Normal saline (2ml/kg) which served as control group, Group-II consisted of rats that are treated with the standard drug Diazepam (1.0mg/kg) and both the groups were treated 30min. prior to the experiment. *Centella asiatica* extract was administered at doses of Group-III 200mg/kg (T1) and Group-IV 400mg/kg (T2) body weight 30 min. before the experiment was conducted. The overall time period of the experiment performed is of 7 days. The behavior coding was done for 5min. in each maze apparatus and following parameters were noted down- time spend in the open arm and time spend in the closed arm. According to these parameters graphs are plotted and their anti-anxiety effect is evaluated. The data is obtained from Elevated plus maze and Elevated zero maze and expressed as mean  $\pm$  SEM. Test-1 (*Centella asiatica* extract 200mg/kg), test-2 (*Centella asiatica* extract 400mg/kg) and Diazepam (1.0mg/kg) administered group was found to have significant decrease in closed arm time as compared to control (2ml/kg normal saline) during behavior coding on the 7th day. It shows that the test-1 (200mg/kg), test -2(400mg/kg) and standard group Diazepam (1.0mg/kg)

spend more time in the open arm than control (2ml/kg normal saline). The values are represented as mean±sem one way anova followed by  $P<0.05$ .

**Table 2:** Effect of control (2ml/kg normal saline), Test-1 (200mg/kg), Test- 2(400mg/kg) and Diazepam (1.0mg/kg) on Elevated Zero Maze

Treatments	Open arm time (7th day) mean ± sem	Closed arm time (7th day) mean ± sem
Control	13.1±0.98	286.8±0.98
Test-1	26.4±1.30	280.0±6.18
Test-2	44.7±1.48	255.2±1.48
Standard	51.4±1.97	248.5±1.97



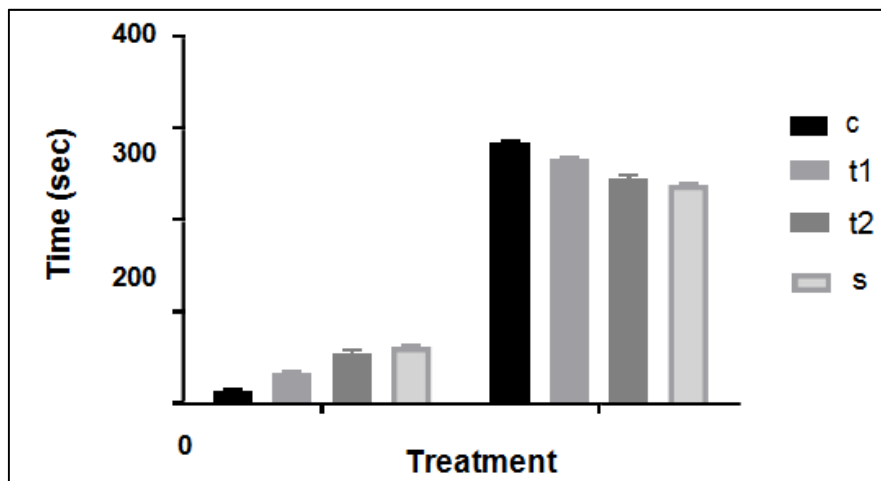
**Fig 3:** Effect of control (2ml/kg normal saline), Test-1 (200mg/kg), Test-2(400mg/kg) and Diazepam (1.0mg/kg) on Elevated Zero Maze

Test-1 (*Centella asiatica* extract 200mg/kg), test-2 (*Centella asiatica* extract 400mg/kg) and Standard Diazepam (1.0mg/kg) administered group was found to have significant decrease in closed arm time as compared to control (2ml/kg normal saline) during behavior coding on the 7th day. It

shows that the test-1 (200mg/kg), test -2(400mg/kg) and standard group Diazepam (1.0mg/kg) spend more time in the open arm than control (2ml/kg normal saline). The values are represented as mean±sem one way anova followed by  $P<0.05$ .

**Table 3:** Effect of control (2ml/kg normal saline), Test 1 (200mg/kg), Test 2(400mg/kg) and Diazepam (1.0mg/kg) on Elevated plus Maze

Treatments	Open arm time 7th day Mean ± sem	Closed arm time 7th day Mean ± sem
Control	14.2±1.32	284.4±1.31
Test-1	33.1±1.86	266.8±1.86
Test-2	54.9±3.65	245.0±3.65
Standard	61.7±1.75	238.2±1.75



**Fig 4:** Effect of control (2ml/kg normal saline), Test 1 (200mg/kg), Test 2(400mg/kg) and Diazepam (1.0mg/kg) on Elevated plus Maze

**Conclusion**

Anxiety is associated with significant physical or mental impairment that disturbs the quality of life among all age

groups. It is the syndrome that is subjective in nature and causes fear from unknown sources. Anxiety is usually transient which presents both physiological and psychological

symptoms in pathological state [31]. The characteristic feature of anxiety is tremor, shortness of breath, palpitations, apprehension, irritability and restlessness. Early detection and appropriate intervention leads to the easy eradication of the pathological conditions of anxiety [32].

The present study is designed to investigate the effect of *Centella asiatica* in rats by Elevated plus maze and Elevated zero maze models respectively. Different parameters are recorded and on the behalf of these parameters it is evaluated that the plant posses the antianxiety effect. The effective increase in the time spent in the open arms and decrease in time spend in closed arms of both the mazes show the antianxiety effect.

The following observations can be summarized on the basis of the results obtained in the present study on Elevated plus maze and Elevated zero maze models:

- Diazepam (1.0mg/kg) group shows significant increase in time spent in the open arms of both the mazes when compared to other groups. The drug belongs to the benzodiazepines category and facilitates GABA neurotransmission for relieving anxiety. In present study it is used as the standard drug.
- *Centella asiatica* extract (200mg/kg) group shows significant antianxiety effect in rats. The rat tries to spend more time in open arms when compared to other groups. The agonistic nature of extract at the 5HT1A receptor could be responsible for the effect elicited by rats.
- *Centella asiatica* extract (400mg/kg) group shows decrease in aversion towards the open arms of the mazes, which is expressed as enhancement in the time spent in open arms of the mazes. The interaction with some of the natural endogenous mediators in the body by the extract shows antianxiety effect. It is reported that the antianxiety effect of the extract is due to the facilitation in the GABA neurotransmission and enhances the chloride conductance. The extract of *Centella asiatica* was investigated for its antianxiety effect in the rats.

From the above data it can be concluded that the ethanolic extract of *Centella asiatica* shows the antianxiety activity and resolves the conflict of avoiding open spaces by the rats in the Elevated plus maze and Elevated zero maze models. It may be used as natural ayurvedic remedy for the treatment of anxiety.

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