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# Antistress activity of ethanolic extract of *Centella asiatica* in foot shock stress induced mouse model

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**ABSTRACT:** **Background:** Stress is considered to cause psychosomatic disorders. *Centella asiatica* is an ancient medicinal plant which has neuroprotective potental. The present study was conducted to explore the antistress activity of *C. asiatica* in foot shock stress induced mouse model. **Aim:** The present study was aimed to evaluate the antistress activity of *C. asiatica*. **Method:** Swiss albino mice were randomly selected and divided into six group that are control, stress control, Diazepam (1 mg/kg) and Imipramine (15 mg/kg), ethanolic extract of *C. asiatica* (EECA) at doses of 100, 200, 400 mg/kg respectively. Evaluation of antistress activity of ethanolic leaves extract of *C. asiatica* has been carried out by using a foot-shock induced stress model which included inescapable electric foot shock (Intensity - 0.8 mA, interval: 10 s, duration: 10 s) delivered through a grid floor once daily for 21 days. Stress results in a significant development of behavioral deficits as assessed on the 21st day on open field test, locomotor activity, elevated plus maze, and tail suspension test. **Result**: Repeated daily administration for 21 days Diazepam and EECA (200 and 400 mg/kg) significantly decreased the motor activity, increased the open arm entries and time spend in open arm in elevated plus maze test and significantly increased number of rearing, number of squares crossed and time spend in centre during 5 min intervals of open field test. Imipramine (15 mg/kg), and EECA 200 and 400 mg/kg also significantly reduced immobility time in tail suspension test. Phytochemical analysis reveals the presence of bioactive substances that are Triterpenoids, Flavonoids, and Glycosides in EECA. **Conclusion**: The EECA showed antistress activity in a dose dependent manner, which may be probably due to the presence of bioactive compounds in EECA. The findings suggest that the EECA has a potential antistress effect that can be explored for therapeutic advantage as an alternative treatment in medical conditions.

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**Keywords:** *Centella asiatica*, Foot-shock model, Behavioral, Antistress, Open Field Test.

## INTRODUCTION:

Stress is the body's non-specific response to any demand for change. The development of mental diseases, such as anxiety or affective disorders is linked to prolonged stress exposure. Stress increases the risk of strokes, heart attacks, hypertension, ulcers, and mental illnesses including depression [1,2]. Strong persistent stress responses might result in tissue damage and illness. Chronic stress can cause hypermetabolism and structural abnormalities in the amygdala, which is responsible for

learning the emotional value of environmental signals and assisting the organism in responding to the threat or challenge posed by these cues [3]. According to the World Health Organization (WHO), mental well-being is a part of health. *C. asiatica* belonging to the family *Umbellifere* (*Apiceae*) is found throughout India growing in moist places up to an altitude of 1800 m [4]. Asiaticoside, brahmoside, asiatic acid, and brahmic acid are pentacyclic triterpenoids found in *Centella*. Centellose, centelloside, and madecassoside are among the other constituents. *C. asiatica* is used in the treatment of minor wounds, hypertrophic wounds, burns, psoriasis, and scleroderma [5].

Stress may also lead to various neurodegenerative disorders and premature aging. There is a current need to develop safe and effective therapeutic medication to combat the adverse effects of stress on the nervous system. Based on the chemical constituent, *C. asiatica* may possess antistress activity.

The present study has undertaken to report the antistress potential of ethanolic extract of *C. asiatica*.

## MATERIALS AND METHODS:

### Reagents and Chemicals:

Diazepam (Neon Laboratories Limited), Imipramine (Osho Pharma Pvt Ltd) and the chemicals used and other solutions were all of analytical grade. All drugs and reagents were prepared immediately before use.

### Collection and Authentication of plant material:

The plant of *C. asiatica* has been procured from Ayurveda shop, Amravati. Authentication of the plant was done from the Botany Department, Vidyabharati Mahavidyalaya, Amravati.

### Preparation of Plant extract:

Fresh leaves were separated from the whole plant and were washed thoroughly with water to remove any dirt particles on the surface of the leaves. The dried leaves of the plant were taken and crushed into a fine powder with the help of a grinder and were kept in air-tight polyethylene bags in the dark until the extraction process. Maceration of 250 g of powder of dried leaves of *C. asiatica* powder in 800 ml ethanol solvent was carried out. The mixture was shaken by an electrical shaker at room temperature for 48 h. The mixture was filtered and solvent was removed on a rotary evaporator. After drying the residue at 70 °C in an electrical oven, a green powder was obtained [6].

### Phytochemical screening:

Qualitative phytochemical investigations were conducted in order to identify the various phytoconstituents.

### Experimental animals:

The experiment was performed on Swiss albino mice, 20 to 25 g which was obtained from the animal house of the Department of Pharmacology, Vidyabharati College of Pharmacy, Amravati. All the animals were acclimatized to animal house prior to use. They are kept in a cage with 12 h light: 12 h dark cycle. Animals were fed on pellets and tap water *ad libitum*. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals (CPCSEA). Permission and approval for animal studies were obtained from Institutional Animal Committee (IAEC) of Vidyabharati College of Pharmacy (Reg No-1504/PO/Re/S/11/CPCSEA), Amravati, and SGB Amravati University.

### Induction of stress by Inescapable foot-shock model:

All animals were habituated for 30 min in their home cages before the start of the experiments. The mice were randomized to 6 groups. Group-I (control) was treated with saline 2 ml/kg, group II (stress control) was treated with saline 2ml/kg, Group III (standard) was treated with diazepam 1 mg/kg i.p., and Group III to V (test group) were treated with different doses of EECA i.e., 100, 200, and 400 mg/kg p.o. respectively (n=6) in each group. Plexiglass chamber (300 × 300 × 350) with a stainless-steel grid floor (4 mm diameter, 9 mm interval) used for foot shock. Inescapable electric foot shocks (intensity: 0.8 mA, interval: 10 s, duration: 10 s) were delivered through a grid floor once daily for 21 days. Each animal was placed in the chamber, after a 2 min adaptation period; the inescapable electric foot-shocks were delivered for a total 5 min [7]. Daily administrations of extract and standard drug were started from the first day of stress procedure. Controls were just placed in the compartment for 30 min without any foot shock. After stress completion, animals were subjected to evaluate antistress activity and their behavioral parameters were recorded as an index of stress.

### Behavioral Assessments:

***Locomotor activity:***

The locomotor activity was measured using an Actophotometer. The movement of the animal cuts off a

beam of light falling on the photocell and a count was recorded and displayed digitally. Motor activity was assessed in terms of total number of counts of light beam interruptions in 10 min. An acquisition period of 5 min was given to each mouse before assessment of motor activity [8].

***Elevated Plus Maze*:**

Elevated plus Maze is a widely and universally accepted paradigm to study anxiety related behaviors in animals. The Plus Maze apparatus consists of two open arms (16 × 5 cm) and two close arms (16 × 5 × 12 cm) and an open roof with the entire maze elevated at a distance of 25 cm from the floor. Lamp was mounted above the apparatus to provide illumination and was kept on throughout the experiment. The drugs as well as vehicle-treated mice were kept individually at the center of the Plus Maze with their head facing towards the open arm. During the 10 min test session, the number of entries into opens and time spent in open arm is recorded [9].

**Open Field Test:**

The open field test is simple and the most frequently used model to study stress induced mice. The apparatus consists of a wooden box (60 × 60 × 30 cm). The base of the box is painted grey or black and is divided into 16 equal squares (15×15 cm). The apparatus was illuminated with 150 to 200 lux in the centre of the open field arena. Mice were placed singly in central position and allowed to explore the apparatus freely. In the 10 minutes session the behavioral 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 area [10].

**Tail Suspension Test:**

Animals were suspended individually by the end of the tail with micropore adhesive tape (approximately 1 cm) with the head 50 cm from the bottom. Mice were suspended for a total of 6 min. During the final 4 min interval of the test, duration of immobility and mobility was recorded. Mice were considered immobile only when they were hung passively and completely motionless [11].

**RESULTS:**

**Phytochemical screening:**

The data of the above table reveals that Alkaloids, Flavonoids, Carbohydrates, Glycoside, Phenolic compounds, Proteins and Amino acids (Table 1) were present in *C. asiactica*.

**Table 1. Preliminary Phytochemical Screening.**

| Phytoconstituents | Test Performed | Inference |
|---|---|---|
| Alkaloid | Wagner's reagent | + |
| Steroids | Sakowaski test | + |
| Phenolic compounds | Ferric chloride Test | + |
| Flavonoid | Shinoda test | + |
| Carbohydrate | Molisch's Test | + |
| | Fehling's Test | |
| Saponin | Soap Formation with water | - |
| Terpenoid | Salkowiski Test | - |
| Glycoside | Keller-killiani test | + |
| Protein and Free Amino Acids | Biuret Test | + |

**(+) and (-) indicates present and absent.**

**Locomotor activity:**

Effects of EECA and diazepam on motor activity of mice exposed to the chronic foot shock was found to be reduce when compared with control and stress control groups (P<0.0001). On day 21 after chronic footshock procedure, the locomotor activity was measured. Daily administrations of EECA and Diazepam were started from the first day of foot shock. The effect on motor activity was elucidated 30 min after the treatment. The effects of EECA and diazepam on motor activity in mice with chronic foot shock (Table 2 and Fig 1) showed that repeated administrations of EECA at doses 100, 200, and 400 mg/kg and diazepam at dose 1 mg/kg significantly reduced motor activity.

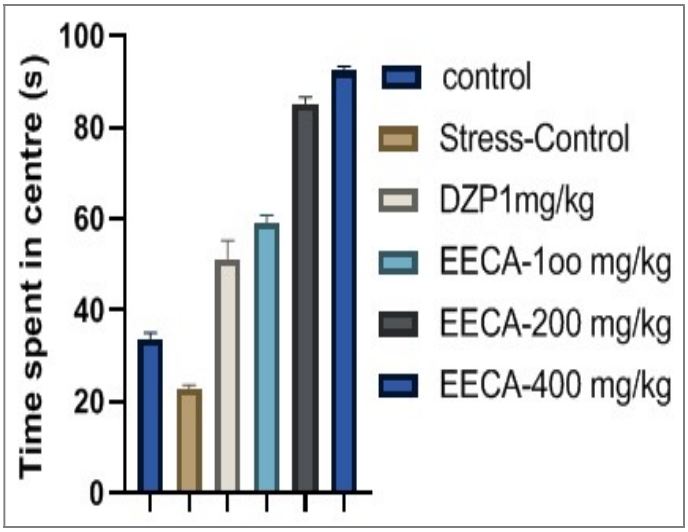


**Fig 1. Effect of ethanolic extract of *C. asiatica* on locomotor activity in mice.**

**Elevated-plus maze:**

Effects of EECA and diazepam on elevated-plus maze test performance of stressed mice significantly increased the number of open arm entries and time spent in open arms. On day 21 after the stress procedure, the animals received the elevated-plus maze test for 10 min. Daily administrations of EECA and diazepam were started from the first day of the stress procedure. The effects on the plus-maze performance were elucidated 30 min after the treatment. As shown in table no 3 and Fig 2 open arm entries and time spent in open arms were significantly increased at doses of 200 and 400 mg/kg of EECA and diazepam at dose 1 mg/kg when compared with control and stress control groups (P<0.0001).

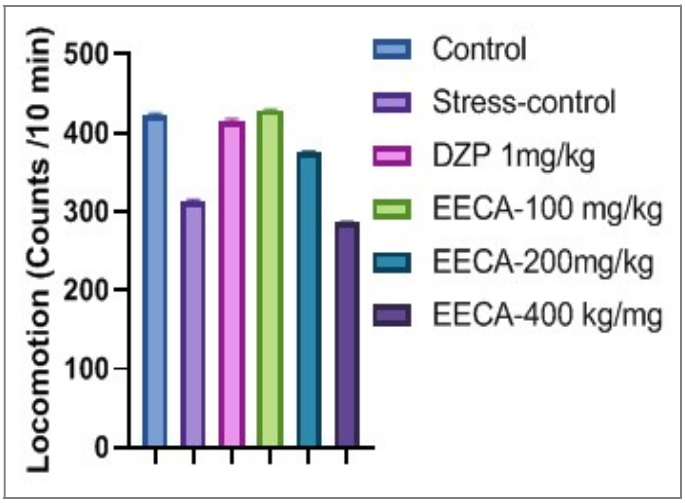


**Fig 2. Effect of ethanolic extract of *C. asiatica* on Elevated-plus maze in mice.**

**Table 2. Effect of ethanolic extract of *C. asiatica* on locomotor activity in mice.**

| Treatment | Dose (mg/Kg) | Locomotion (Counts/10 min) |
|---|---|---|
| Control | 2 ml/kg ip | 423.6±1.2 |
| Stress-Control | 2 ml/kg ip | 314±1.4 |
| Standard (Diazepam) | 1mg/kg ip | 415.8±2.0 |
| EECA | 100 mg/kg po | 429±1.0 |
| EECA | 200 mg/kg po | 376±0.7071*** |
| EECA | 400 mg/kg po | 286.4±0.9274*** |

**Results are expressed as mean ± SEM (n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Tukey test. *** indicates P<*0.0001* when compared to control and stress control group.**

**Table 3. Effect of ethanolic extract of *C. asiatica* on Elevated plus maze Test in mice.**

| Treatment | Dose (mg/kg) | Open arm time (s) | Open arm entries (10 min) |
|---|---|---|---|
| Control | 2 ml/kg ip | 31.2 ±0.8602 | 14 ±0.7071 |
| Stress-control | 2 ml/kg ip | 36 ± 0.7071 | 7.6 ±0.9274 |
| Standard (Diazepam) | 1mg/kg ip | 76 ±0.7071*** | 12.4 ± 0.9274*** |
| EECA | 100 mg/kg po | 26.8 ± 1.068 | 18 ±0.9274 |
| EECA | 200 mg/kg po | 55 ±1.000 | 23.8 ±0.8602 |
| EECA | 400 mg/kg po | 66.2 ±1.068*** | 34 ±0.7071*** |

**Results are expressed as mean ± SEM (n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Tukey test. *** indicates P<*0.0001* when compared to control and stress control group.**

**Table 4. Effect of ethanolic extract of *C. asiatica* on open field test in mice.**

| Treatment | Dose (mg/kg) | Time spent in centre (s) | Number of rearing | Number of square crossed (s) |
|---|---|---|---|---|
| Control | 2 ml/kgip | 33.8 ±1.241 | 5.2±0.8602 | 41.2±1.158 |
| Stress-Control | 2 ml/kg ip | 22.8±0.8602 | 3.8±0.5831 | 38.0±1.068 |
| Standard (Diazepam) | 1mg/kg ip | 51.2±4.116 | 7±0.7071 | 53±0.7071 |
| EECA | 100 mg/kg po | 59.2±1.562 | 4±0.7071 | 54±0.7071 |
| EECA | 200 mg/kg po | 85.2±1.393 | 5.8±0.6633 | 57±0.8602 |
| EECA | 400 mg/kg po | 92.4±0.9274*** | 6.4±0.5099*** | 60±0.8602*** |

**Results are expressed as mean ± SEM (n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Tukey test. *** indicates P<*0.0001* when compared to control and stress control group.**

**Table 5. Effect of ethanolic extract of *Centella asiatica* on Tail suspension test in mice.**

| Treatment | Dose (mg/kg) | Duration of Immobility (s) |
|---|---|---|
| Control | 2 ml/kg i.p. | 113 .6 ± 0.9274 |
| Stress-control | 2 ml/kg i.p. | 121.2 ± 0.8602 |
| Standard (Imipramine) | 15 mg/kg p.o. | 82.6 ± 1.030*** |
| EECA | 100 mg/kg p.o. | 81.0 ±0.7071 |
| EECA | 200 mg/kg p.o. | 75.2 ±0.8602 |
| EECA | 400 mg/kg p.o. | 70.0 ±1.158*** |

**Results are expressed as mean ± sem(n=6). Data was analysed by one way analysis of variance (ANOVA) followed by Tukey test. *** indicates *P<0.0001* when compared to control and stress control group.**

**Open Field test:**

Effects of EECA and diazepam on Open field test performance of stressed mice significantly increases the time spent in centre and increased the number of rearing and number of square crossed. On day 21 after stress procedure, the animals received the Open field test for 10 min. Daily administrations of EECA and Diazepam were started from the first day of stress procedure. The effects on the Open field test performance were elucidated 30 min after the treatment. As shown in Table 4 and Fig 3 at doses of 200 and 400 mg/kg of EECA and diazepam at dose 1mg/kg showed increased time spent in centre and increase the number of rearing and number of square crossed when compared with control and stress control group (P<0.0001).

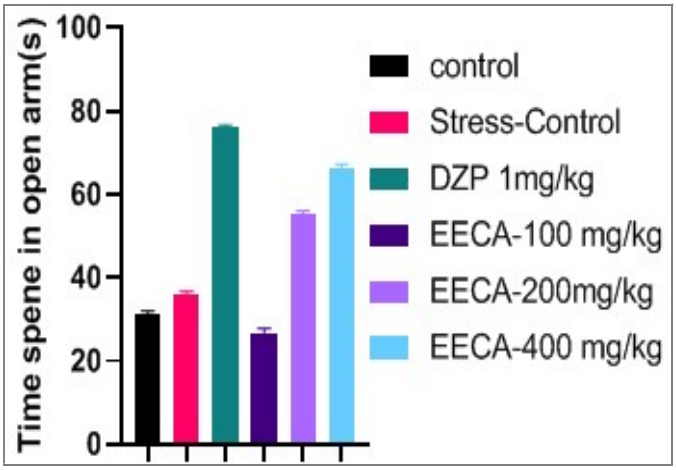


**Fig 3. Effect of ethanolic extract of *C. asiatica* on Open field test in mice.**

Effects of EECA and imipramine on Tail suspension test performance of stressed mice significantly decrease the duration of immobility period. On day 21 after stress procedure, the animals received the Tail suspension test for 10 min. Daily administrations of EECA and imipramine was started from the first day of stress procedure. The effects on the Tail suspension test performance were elucidated 30 min after the treatment. As shown in table no 5 and Fig 4 at doses of 200 and 400 mg/kg of EECA and imipramine at dose 15 mg/kg significantly decrease the duration of immobility when compared with control and stress control group (P<0.0001).

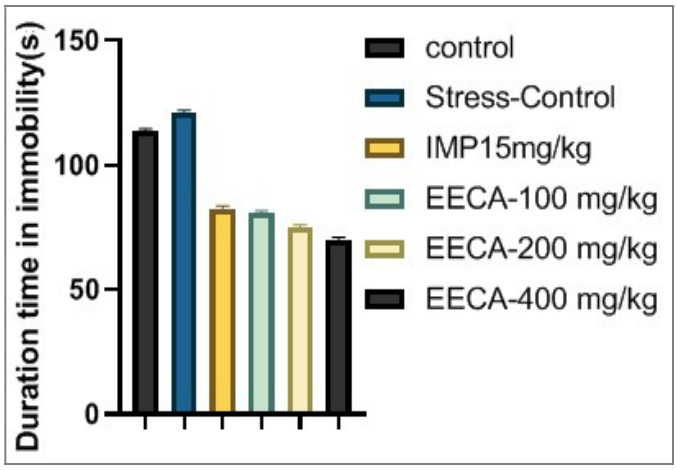


**Fig 4. Effect of ethanolic extract of *C. asiatica* on Tail suspension test in mice.**

**DISCUSSION:**

An imbalance between sympathetic and parasympathetic nervous systems is responsible for stress syndromes. A large proportion of all diseases are believed to occur due to stress closely associated with modernization of life [12] Individual differences in handling psychological distress and stress mediated contexts were prominent during the pandemic [13,14].

Despite the availability of many pharmaceutical products for the treatment of stress in the market, their successes were limited by the presence of several adverse effects. Due to reported side effects of available antistress drugs, focus has been shifted towards natural products as the new sources of antistress agents. Various plants have been investigated based on traditional knowledge of their pharmacological qualities and confirmed to be beneficial in treating and managing stress as a result of the growing interest in natural medicine. This study found that mice

exposed to adverse footshocks not only developed long-term anxiety, but also developed a particular avoidance of the setting associated with the aversive foot shock. Chronic stress is generally considered as a key risk factor for the development of a variety of human ailments. Specifically, anxiety and depressive disorders have been frequently associated with stressful life events. The stress system's activation causes behavioral and peripheral changes that help to restore homeostasis and improve stress coping. A lack of adaptability to high demands, on the other hand, can lead to pathological disorders like sadness and anxiety. The present study investigated the antistress effect of ethanolic extract of *C. asiatica* on foot shock induced stress in mice. In the present study, EECA at the dose 200 and 400 mg/kg was found to have an antistress effect on locomotor activity, elevated plus maze, open field test and tail suspension test. In the present study, after repeated daily administration for 21 days Diazepam dose of (1 mg/kg) and EECA 200 and 400 mg/kg significantly decreased the motor activity, increased the open arm entries and time spend in open arm in elevated plus maze test and significantly increased number of rearing, number of squares crossed and time spend in centre during 5 min intervals of open field test. Imipramine (15 mg/kg), and EECA 200 and 400 mg/kg also significantly reduced immobility time in tail suspension test. The central nervous system's neurochemical pathways have been shown to play a key role in the control of stress responses, such as reducing serotonergic transmission in the prefrontal cortex, which has been linked to the pathophysiology of depression and anxiety. Several lines of evidence demonstrate that persistent stress degrades monoamines such as serotonin, noradrenalin, and dopamine, which may be the cause of anxiety and behavioural depression. In the present study, Pre-treatment with *C. asiatica* leaf extracts may decrease stress-induced depletion of norepinephrine and dopamine levels, allowing animals to cope better with stressful situations and lowering anxiety and sadness. Earlier reports on the chemical constituents of various plants and their pharmacology suggest that plants containing flavonoids and tannins possess activity against many central nervous system disorders. The phytochemical screening of EECA showed presence of bioactive compounds. The antistress activity may be probably due to the presence of bioactive compounds like Triterpenoids, Flavonoids, Glycosides, and Tannins in the EECA. It might be possible that these bioactive compounds in EECA are involved in increasing the levels of norepinephrine, dopamine and serotonin levels thus reducing stress related anxiety and depression.

## CONCLUSION:

The finding suggests that the ethanolic extract of *C. asiatica* has a potential antistress effect that can be explored for therapeutic advantage as an alternative treatment in medical conditions. The antistress activity is probably due to the presence of bioactive compounds like Triterpenoids, Flavonoids, and Glycosides in the EECA. Further studies are required to confirm the exact mechanism and isolation of the bioactive compounds involved in the extract.

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