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A preclinical study of *Elettaria cardamomum* for its antianxiety activity in Wistar albino rats

Prathima K Shetty[1], Megha Rani N[2], Rejeesh EP[3], Rao SN[4]

[1]Medical Data Review Manager, Centralised Monitoring Operations, Labcorp Drug Development India Private Limited, Bengaluru, [2]Associate Professor, Department of Pharmacology, [4]Ex-Professor, Department of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru, Karnataka, [3]Professor, Department of Pharmacology, Mount Zion Medical College, Adoor, Kerala, India

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ABSTRACT

Background: *Elettaria cardamomum*, commonly known as cardamom, is one of the most widely used spices worldwide and is conventionally well known for its effects on the central nervous system (CNS) as a food additive. **Aims and Objectives:** This study was carried out to assess the ethanolic extract of *E. cardamomum* (EEEC) for its CNS activity in rats. The purpose of this study was to validate the traditional use of *E. cardamomum* as an antianxiety agent. **Materials and Methods:** The elevated plus maze and light–dark arena models were used to evaluate its anxiolytic activity. The open field test and actophotometer were used for assessing its effect on locomotor activity. The experiments were performed in Wistar albino rats of either sex after grouping the animals into three different groups. Twelve animals per group were used. Distilled water (10 mL/kg) was used as Control and Diazepam (1 mg/kg) were used as standard drugs for the respective tests. **Results:** EEEC (at a dose of 100 mg/kg) has shown anxiolytic and reduced locomotor activity. **Conclusion:** The results of this study indicate that EEEC has anxiolytic and sedative effects, similar to benzodiazepine and possibly similar mechanisms.

Key words: Actophotometer; Anti-anxiety; Ethanolic extract; *Elettaria cardamomum*; Elevated plus maze; Light and dark arena; Locomotor activity; Open field; Wistar albino rats

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INTRODUCTION

Over recent years, modern medicine and medical technology have advanced in leaps and bounds. Despite this, if we have to meet the health-care requirements of our increasing population, a strategy has to be implemented to procure cheaper and more effective medications.[1]

It is observed from different parts of the world, including ours, that there cannot be a better alternative than "herbal drugs." History tells us that "herbs" have saved numerous lives.[1]

Our country is rich in flora. If we dig into the ancient texts, the number of uses of some common herbs will surprise us. However, their uses and efficacy need validation. These herbal drugs have to be researched using modern analytical techniques. This can lead to the discovery of new chemical entities which can cure a large number of ailments. However, the cost of such research is not comparable to the billion dollars spent on the synthesis of a new molecule in the conventional system.[1,2]

The other advantage of using principles from the alternative system of medicines is that apart from the fact that they would be cheap (as they are available in abundance), people have a belief that they have lesser side effects. Moreover, it would probably be easier to convince a person suffering from an ailment to take a substance that they have come across on a day-to-day basis.[3] This is especially important in psychopharmacology as one of the main problems with patients with mental illness (unlike other diseases) is that they lack insight into the disease and hence poor compliance.[4] The medications used (in the allopathic system of medicine) to combat them come with their huge

Address for Correspondence:
Dr. Megha Rani N, Associate Professor, Department of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, India. **Mobile:** +91-9481506337. **E-mail:** ranimegz@gmail.com

array of dreaded side effects. There is a need to explore the principles used in the time-tested traditional system of medicines for an alternative with fewer side effects.[3]

Elettaria cardamomum Maton, the "Queen of Spices" is one such common spice which has shown various medicinal properties in addition to its wide use for culinary purposes. Its variety of uses is mentioned in various texts on ayurveda and unani System of medicine.[5] Avicenna's book on aromatherapy mentions its use in nervous exhaustion.[6]

E. cardamomum is also a constituent of numerous medicinal preparations such as Mentat,[7] Brahmi Rasayan,[8] and Unmadnashak Ghrita[9]. Moreover, one of the articles has reported its sedative effect and has concluded that its potential anticonvulsant property has to be researched further.[10] Hence, it would be worthwhile to screen *E. cardamomum* for its central nervous system (CNS) activities.

Aims and objectives

The aim of the study was to assess the antianxiety activity of ethanolic extract of *Elettaria cardamomum* using elevated plus maze and light and dark arena and locomotor activity using open field test and actophotometer using wistar albino rats.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, Yenepoya Medical College, Mangalore, after getting approval from the Institutional Animal Ethics Committee.

Materials

Experimental animals used in the study

Healthy adult Wistar Albino rats of either sex weighing 150–200 g, (347/PO/ReBi-S/Rc-L/01/CPCSEA) were used for the study. The rats were housed in clean polypropylene cages, six rats in each cage, under standard housing conditions maintained at a room temperature of 24±2°C with a 12 h light and dark cycle. They had access to standard chow containing fat at 4.15%, proteins at 21.15%, carbohydrates at 4%, and water ad libitum. The rats were marked for identification and were allowed to acclimatize to the laboratory conditions for 1 week before the experiments. They were handled with care according to the CPCSEA guidelines.[11]

Plant material

E. cardamomum fruits were procured from a plantation in Mudigere, Chikmanglur, Karnataka. It was authenticated by a plant taxonomist from Mangalore University.

Dried fruits (weight- 250 g) were ground with the outer shells to obtain a coarse powder using an electric mixer. This was subjected to Soxhlet extraction[12] using ethanol (90%) as solvent maintained at 60°C for 20 h. The ethanolic extract of *E. cardamomum* (EEEC) was made free from the solvent using a rotary evaporator (Yield - 7%).

Equipment, drugs and chemical solutions - Elevated plus maze, light–dark arena, oral feeding tube/gavage, weighing balance, cotton, spirit, gloves, stopwatch, distilled water, diazepam– obtained from Rajesh Chemicals, Mumbai.

Methodology

Assessment of anxiolytic activity

The study was carried out using two animal models which are widely validated for measuring anxiety in rodents – Elevated plus maze and light–dark arena exploration tests. A total of 36 adult Wistar albino rats of either sex were used in the study. Animals were divided into three groups of twelve animals each. Group 1 (Control group) received distilled water (10 mL/kg), Group 2 (Standard group) received Diazepam (1 mg/kg)[13], and Group 3 (Test Group) received EEEC (100 mg/kg).[10] The control, standard drug, and the test compound were administered orally, daily for 10 days and the experiment was carried out on the 10th day 60 min after administering the drug.

Elevated plus maze[14-16]

The plus-maze (cross-shaped maze) consists of four arms, two enclosed arms, and two open arms that are interconnected by a central platform. The maze is suspended 50 cm above the room floor. The animal was placed on the central platform, facing one of the enclosed arms, and observed for 5 min. During the 5 min test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, were recorded. An entry was defined as the presence of all four paws in the arm. The arms were cleaned of the feces each time after the 5 min of exploration before placing a new animal to inhibit olfactory clues from the feces of the previous animal.

Light–dark arena[14-16]

The maze is divided into two parts, 1/3 with opaque walls and a cover (dark compartment), whereas the remaining 2/3 is open and illuminated (light compartment). The opening between the two compartments permits the rat to move from one side to another. The rats were released in the light compartment and observed for 5 min. During that time, the time spent in light and dark compartments and the number of transitions was recorded.

Assessment of locomotor behavior

Open field apparatus[17-19]

This is a simple test to assess mobility or activity in the novel open field and the emotionality of the animal. The motor activity of rats will be measured in a plexiglas open field maze (3ft×3ft×1ft deep) marked off into 36

Table 1: Elevated plus maze model - effect of different treatments on the rats

Groups	Treatments (dose/kg)	Time spent (s)		Number of entries
		Open arm	Closed arm	Open arm
I Control	Distilled water (10 mL/kg)	81±11.37	217.91±11.26	5.3±1.23
II Standard	Diazepam (1 mg/kg)	162.75±7.78**	137.25±7.78	11.16±1.19**
III Test	EEEC (100 mg/kg)	133.58±15.99**	167.25±15.94	7.0±1.27*

Values expressed as mean±SD, n (number of rats)=12, symbol * and **Significant values at P<0.05 and P<0.01, respectively, versus control; ANOVA followed by Dunnet's multiple comparison test. SD: Standard deviation, ANOVA: Analysis of variance

Table 2: Light dark arena model – effect of different treatments on the rats

Groups	Treatments (dose/kg)	Time spent in seconds	
		Light	Dark
I - Control	Distilled water (10 mL/kg)	77.5±7.96	227.5±17.720
II - Standard	Diazepam (1 mg/kg)	144.25±19.46**	152.41±20.92
III - Test	EEEC (100 mg/kg)	120.25±8.76**	179.75±8.76

Values expressed as mean±SD, n (number of rats)=12, symbol **Significant values at P<0.05 and P<0.01, respectively, versus control; ANOVA followed by Dunnet's multiple comparison test. SD: Standard deviation, ANOVA: Analysis of variance

Table 3: Actophotometer – effect of different treatments on the rats

Groups	Treatments (dose/kg)	Total number of movements
I	Distilled water (10 mL/kg)	245.75±24.49
II	Diazepam (1 mg/kg)	125.25±10.33**
III	EEEC (100 mg/kg)	166.58±20.16**

Values expressed as (mean±SD) using actophotometer in rats (n=12), symbol **Significant values at P<0.05 and P<0.01, respectively, versus control; ANOVA followed by Dunnet's test. SD: Standard deviation, ANOVA: Analysis of variance, EEEC: Ethanolic extract of *Elettaria cardamomum*

equal segments with black paint. Normally a rat, placed in a brightly lit novel field explores the periphery of the apparatus. Mobility or activity in the open field will be quantified by keeping account of the number of squares (peripheral or central) crossed by the rats in the open field, or the time spent in those areas along with evaluation of autonomic function, namely defecation.

Actophotometer[17-19]

The locomotor activity can be easily measured using an actophotometer, which has a square arena in which the animal moves. This apparatus operates on photo-electric cells, which are connected in a circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded.

RESULTS

Table 1 shows the results of elevated plus maze. Results were expressed as mean±SD/SEM. Statistical analysis of the data was done using a one-way analysis of variance followed by Dunnet's multiple comparison test.[20] P<0.05 was considered significant.

Anxiolytic activity

Elevated plus maze

Group II (Diazepam group) showed a significant increase in the time spent in the light area ($p < 0.01$) when compared to Group I (Control group) as shown in Table 2. Similarly, Group III (EEEC) showed a significant increase in time spent in the light area ($p < 0.05$) when compared to the control group.

Light–dark model

Table 3 shows the result of actophotometer and shows that Group II (Diazepam group) & Group III (EEEC) showed a significant decrease in the mean movements in the Actophotometer ($p < 0.01$) when compared to Group I (Control group).

Locomotor behavior

Actophotometer

Group II (Diazepam group) and Group III (EEEC) showed a significant decrease in the mean movements in the actophotometer (P<0.01) when compared to Group I (Control group).

Open field test

Table 4 shows the results of open field study and shows that Group II (Diazepam group) & Group III (EEEC) showed a significant decrease in the mean movements in the open field test ($p < 0.05$) when compared to Group I (Control group).

DISCUSSION

The present study was carried out to evaluate the anxiolytic activity, the locomotor activity of EEEC at the dose of 100 mg/kg, and the detailed discussion is as follows:

Anxiolytic activity

The anxiolytic activity was tested in two different animal models, the elevated plus maze and the light–dark arena, and was compared with diazepam, a standard anxiolytic drug.

Table 4: Open field test – effect of different treatments on the rats

Groups - (n=12)	Treatments - (dose/kg)	Total number of squares crossed
I - Control	Distilled water (10 mL/kg)	38.75±8.76
II - Standard	Diazepam (1 mg/kg)	82.66±6.55**
III - Test	EEEC (100 mg/kg)	57.78±29.36**

Values expressed as (mean±SD) using open field apparatus in rats (n=12), symbol **Significant values at P<0.05 and P<0.01, respectively, versus control; ANOVA followed by Dunnet's test. SD: Standard deviation, ANOVA: Analysis of variance, EEEC: Ethanolic extract of *Elettaria cardamomum*

The elevated plus maze is considered an etiologically valid animal model of anxiety. It evaluates based on 3 anxiogenic factors – novelty, height, and exploratory behavior. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The reduction in entry and time spent in open arms are indications of high levels of fear or anxiety. The number of entries and time spent in the open arms are increased by anxiolytics and reduced by anxiogenic agents.

The light/dark exploration test is based on the natural aversion of rats to brightly lit places. Anxiolytics reduce the natural aversion to light and increase the time spent in the lit compartment. Our study showed that *E. cardamomum* ethanolic extract in the dose of 100 mg/kg, given orally produced a significant increase in the time spent in the well-light box and a decrease in the time spent in the dark box when compared with the control group and results comparable with the standard drug, thus demonstrating its anxiolytic-like activity.[14,15] In elevated plus maze model also showed a significant increase in both the time spent in open arms and the entry into open arms at the same dose of EEEC, suggesting anxiolytic activity, which is in line with the results of light and dark arena.

Locomotor activity

The open field test and the actophotometer were used to test the effect of EEEC on locomotor activity.

Hall (1934) originally described the open field test for the study of emotionality in rats.[21] The open field test is now one of the most popular procedures in animal psychology (Belzung, 1999).[22] Different versions of open field maze are available, differing in the shape of the environment. However, a simple version was used in our study, where the total number of squares crossed in 5 min when placed in the box was recorded manually and a reduction in the number of squares crossed gives the sedative or CNS depressant effect of the drug.

EEEC significantly decreased the number of boxes crossed or movements when compared to the control group indicating the sedative property of the extract. Similar results were obtained in the actophotometer, which is a closed box in which the movements of the animal are recorded photoelectrically.

Preliminary phytochemical analysis done by us showed the presence of alkaloids, tannins, terpenoids, phytosterols, flavonoids, carbohydrates, and protein.[23] Studies have shown that some flavonoids bind with high affinity to the benzodiazepine site of the GABA receptor. Benzodiazepines also bind to GABA receptors and produce anti-anxiety effects. It can be postulated that the flavonoids present in EEEC can be responsible for the antianxiety effect[24] exhibited by this plant in our study.[25] Previous studies done on this constituent have shown a dose-dependent sedative effect too. A study done by Yaser Masoumi-Ardakani et al., on methanolic extract of *E. cardamomum* at 3 doses of 200, 400, and 800 mg/kg found that the extract exhibited significant antianxiety activity at the dose of 400 mg/kg and skeletal muscle relaxant activity at 800 mg/kg. They attributed the above-mentioned activity to the presence of quercetin in the extract; however, they mentioned that the data obtained are insufficient and further exploration is required to confirm the same.[26] Our study confirms the antianxiety effect even at a lower dose of 100 mg/kg with ethanolic extract.

However, further studies are required to confirm this finding. Thus, it has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism/mechanisms of action of the extract, as well as the active substance/substances responsible for its biological actions, is necessary.

Limitations of the study

The main limitation is that it is a preliminary study and further studies to identify and isolate the primary active compound responsible for the activity will be more value added.

CONCLUSION

The results of this study indicate that EEEC has anxiolytic and sedative effects, similar to benzodiazepine and possibly similar mechanisms.

Further studies to explore and isolate active constituents which are responsible for these effects will lead us to new compounds with therapeutic potential. However, more studies on both the dose-dependent effect and studies designed to elicit the precise mechanism of action as well as the pharmacokinetic parameters are needed to get a better understanding of this compound.

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Authors' Contributions:
PKS- Definition of intellectual content, concept, literature survey, prepared a first draft of the manuscript, conducting the experiment, data analysis, manuscript preparation; **MRN-** Conducting the experiment, data analysis, manuscript preparation, editing, manuscript revision and submission of the article; preparation of tables; coordination; **REP-** Statistical analysis and interpretation; review manuscript; **RSN-** Review manuscript, editing, and revision.

Work attributed to:
Department of Pharmacology, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangaluru, Karnataka, India.

Orcid ID:
Dr. Prathima K Shetty - https://orcid.org/0009-0006-6932-1963
Dr. Megha Rani N - https://orcid.org/0000-0003-4270-755X
Dr. Rejeesh EP - https://orcid.org/0000-0001-9927-9221
Dr. Rao SN (Retired) - https://orcid.org/0000-0001-7007-5428

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