EVALUATION OF ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF CUMINUM CYMINUM IN RODENTS

ZAKKI ARSHAD[1], AHMED UMER SOHAIB*[2], TASSAWER-MERAN[2], MUHAMMAD OSAMA[2], SAMIE ULLAH[2], ANUM YASMEEN[3], SYED IMRAN HAIDER[4]

1. Lahore College for Women University, Lahore
2. The Superior University, Lahore
3. Punjab University, Lahore
4. CMH Lahore Medical and Dental College, Lahore

ABSTRACT

The present study aimed to investigate the anxiolytic activity of ethanolic extract of Cuminum cyminum (cumin) in healthy male albino mice. Furthermore, mechanism of action involve in their anxiolytic activity was also evaluated. Cuminum cyminum (C. cyminum) ethanolic extract were administered i.p in doses of 100, 200, 300 mg/kg to healthy mice. Diazepam 0.5 mg/kg was used as standard drug and normal saline as negative control. Anxiolytic activity of control, standard, C. cyminum were evaluated using Elevated plus maze and Light and Dark compartment test. C. cyminum extract at dose 200mg/kg had maximum anxiolytic effect by significant increase in percentage of open arm entries (C. cyminum mean ± SEM value 34.60 ± 4.22, p value 0.04) and time spent in open arms (C. cyminum mean ± SEM value 53.40 ± 26.18, p value 0.036) in elevated plus maze and significant increase in percentage of time spent (C. cyminum mean ± SEM value 47.05 ± 5.47, p value 0.041). When pretreated with flumazenil (GABA receptor antagonist) C. cyminum (200mg/kg) had significant decrease in anxiolytic activity by significant decrease in percentage of open arm entries (C. cyminum mean ± SEM value 7.08 ± 4.39, p value 0.000), time spent in open arms (C. cyminum mean ± SEM value 4.00 ± 2.45, p value 0.046) in elevated plus maze, number of entries (C. cyminum mean ± SEM value 2.20 ± 0.37, p value 0.000 and C. sativum mean ± SEM value 8.40 ± 1.40, p value 0.001) and percentage of time spent (C. cyminum mean ± SEM value

8.06 ± 2.99, p value 0.000) in light compartment of light and dark compartment test. It is concluded that ethanolic extracts of C. cyminum has anxiolytic activity and GABAA receptors in brain are involve in its anxiolytic action.

Keywords: Anxiolytics, Cuminum cyminum, GABA receptors, ethanolic extract.

INTRODUCTION

Anxiety is a reaction categorized by feelings of tension, worried thoughts, and physical alterations like increased blood pressure. Patients with anxiety usually have frequent intrusive thoughts. General symptoms of anxiety are feelings of panic, fear, nervousness, sweating, shortness of breath, heart palpitations, trembling [1]. Gamma Amino Butyric Acid receptors ($GABA_A$ receptors) in central nervous system are involved in the anxiety [2]. Benzodiazepine and selective serotonin reuptake inhibitors are two classes of drugs which are mostly use for the treatment of anxiety. BZD binds at $GABA_A$ receptors, increases the frequency of opening of chloride ion channel and causes hyperpolarization that has inhibitory action on conduction of nerve impulse. Due to several side effects and dependence on this class of drug, herbal medicines have greater interest from their better compliance and lower side effects [3].

Flavonoids are present in different vegetables, fruits, and beverages (green tea, wine). They have flavan ring (Fig 1.1) which is structurally resemble to GABA (fig1.2) inhibitory neurotransmitter of brain [4]. Flavonoids, terpenes, and other related substances affect the function of the GABA receptors [5].

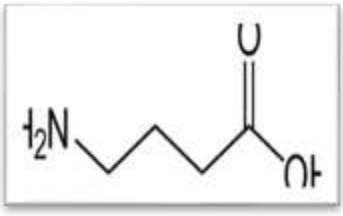

Fig 1.1 [6]

Fig 1.2 [7]

Cuminum cyminum commonly known as "*zeera"*. It is an annual herbaceous plant

and belongs to family Apiaceae (shown in Fig 1.3 and Fig 1.4). Different constituents present in white cumin seeds are alkaloids, flavonoids, essential oils, glycosides, proteins, steroids, phenolic compounds and tannins, fixed oils, fats and reducing sugars [8]. Cumin aldehyde is the highest active constituent and Limonene, eugenol, α pinenes, β pinenes, cuminosid A and cuminoside B are some other minor constituents in cumin oil. Different pharmacological activities of cumin have been found till now which includes antispasmodic, appetite stimulant, antihyperglycemic activity, antimicrobial activity and hypolipidemic [9]. Anxiolytic activity is not studied up till now but in Ayurvedic medicine, powder of cumin seeds is taken with a ripe banana at bedtime to induce sleep. Tea of cumin seeds is also used to induce sleep in traditional medicine [10].

Taxonomical Classification

Table 1.1: Taxonomical lineage of *Cuminum cyminum*

Fig 1.3 *Cuminum cyminum*

[www.henriettesherbal.com]

Fig 1.4 Refined form of *Cuminum cyminum* [11]

MATERIAL AND METHODOLOGY

Chemicals

Normal saline, Diazepam (Valium injection 10mg/ 2ml) and Flumazenil (Anexate injection 1mg/10ml) were used.

Models: Elevated plus maze (EPM)

The elevated plus maze was used for spontaneous detection of anxiolytic behavior [12].

Light and Dark compartment Test

The apparatus was used to assay unconditioned anxiety responses in rodents [13].

Plant Extract

Seeds of *Cuminum cyminum* was taken from the local market. Seeds of *C. cyminum* were collected and dried at 35–40°C for 1–2 days. Dried seeds were crushed in grinder to have fine powder. Powder was defatted using petroleum ether and continuously extracted with ethanol in Soxhlet apparatus. After filtration of extract, it was concentrated on rotary evaporator.

Test for Detecting Flavonoids

Crude extract was added in 2ml of 2% solution of NaOH. An intense yellow color was form which turned colorless on addition of few drops of dilute acid which indicated the presence of flavonoids.

Animal preparation

Healthy mice (Swiss albino Male 25–35 g) were purchased. The mice were maintained at suitable nutritional and environmental conditions throughout the experiment in animal house of Department of Pharmacy, LCWU. The mice were housed under standard laboratory conditions for acclimatization period of seven days prior to perform the experiment.

Study groups

I. Group I (Normal saline 0.1 ml/10g)
II. Group II (Diazepam 0.5 mg/kg)
III. Group III (Cumin extract 100mg/kg)
IV. Group IV (Cumin extract 200mg/kg)
V. Group V (Cumin extract 300mg/kg)

There was a separate group for each dose and each anxiety testing model. Each group was having 5 male albino mice.

Inclusion & Exclusion criteria

Inclusion criteria	Exclusion criteria
Swiss male albino mice Mice of weight 25-35g Acclimatized mice	Swiss female albino mice Mice having weight less than 25g and greater than 35 g

Administration of drugs for anxiolytic activity testing

Different concentration doses were prepared freshly on experiment days and administered intraperitoneally (i.p.).

Dose volume was administered according to mice body weight (0.1 ml/10 g) and readings were taken 30 minutes after administration.

Administration of drugs for mechanism of action determination

Animals were pretreated with Flumazenil 30 minutes before they were treated with doses of normal saline, Diazepam, and *C. cyminum* were tested separately on the EPM and light and dark compartment after 30 minutes of administration of doses. Different concentration doses were prepared freshly on experiment days and administered intraperitoneally (i.p.). Dose volume was administered according to mice body weight (0.1 ml/10 g).

Elevated plus maze:

Each mice was placed separately at the center of elevated plus maze with the head of mice towards an open arm and observation was done for 5 minutes and the number of entries in open arms, closed arms and time spent in each arm was noted. The percentage of open arm entries taken as follows:

Percentage of open arm entries = Number of open arm entries ÷ Total entries × 100

Light and dark compartment test

A mice was placed in the center of light compartment and observation was done for 5 minutes for the number of entries in light compartment and percentage of time spent in light compartment. Percentage of time spent in the light compartment taken as follows:

Percentage of time spent in light compartment = Time spent in light compartment ÷ Total time (300 seconds) × 100

Statistical analysis

Data of EPM and light and dark compartment test of Normal Saline and Diazepam treated group was statistically analyzed by using independent t-test. Data of *C. cyminum* was extract was evaluated by one way ANOVA. Comparisons between individual groups were done by using post hoc comparison (Dunnett t test). SPSS 17.0 software was used for statistical analyses. P value less than 0.05 were taken as significant level.

RESULTS

Effect of Different doses of *Cuminum cyminum* extract on elevated plus maze (EPM)

Different doses of ethanolic extract of *C. cyminum*, control (Normal Saline) and standard (Diazepam) were administered intraperitonially to healthy male albino mice and their anxiolytic activity was measured after 30 minutes. Mice were placed in the center of EPM, head of the mice towards open arm and observation time was 5 minutes to note the number of entries in closed arm and open arm. Diazepam at dose 0.5 mg/kg and ethanolic extract of *C. cyminum* at dose 200 mg/kg had significantly increase (*P* less than 0.05) in percentage of open arm entries (fig 4.5) and time spent on open arms (fig. 4.6) compared to control group (Normal Saline). Doses 100mg/kg and 300mg/kg of *C. cyminum* extract showed no significant increase in percentage of open arm entries and time spent on open arms.

Table 4.1: Effect of different doses of

Groups	Treatments	Doses	Percentage of entries in open arm	Time spent in open arm (seconds)
I	Normal saline	0.1 ml/10g	18 ± 5.33	8.60 ± 3.44
II	Diazepam	0.5 mg/kg	(50.22 ± 7.18) *	(79.60 ± 25.6) *
III	C. cyminum extract	100mg/kg	15.80 ± 6.40	8.60 ± 3.04
IV	C. cyminum extract	200mg/kg	(34.60 ± 4.22) *	(53.40 ± 26.18) *
V	C. cyminum extract	300mg/kg	14.17 ± 3.56	13 ± 7.53

Cuminum cyminum extract on elevated plus maze

*: showing significant value, P < 0.05. Values are expressed as Mean ± SEM, (n=5

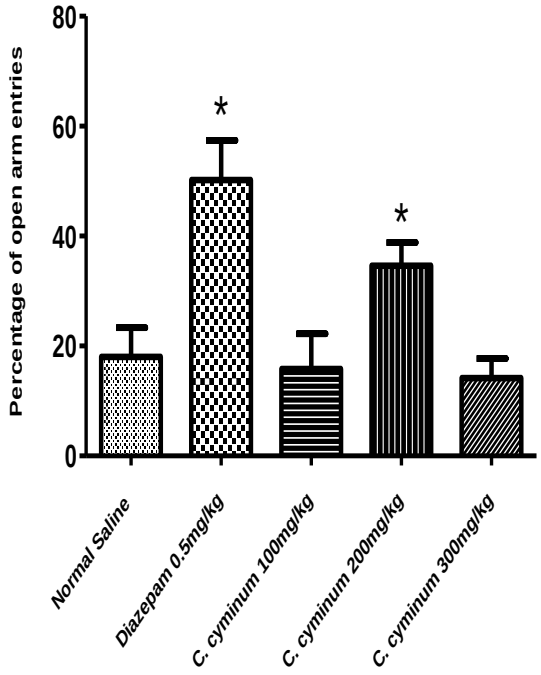

Fig 4.5 The effect of diazepam and ethanolic extract of C. cyminum seeds (100, 200, 300 mg/ kg) on the percentage of open arm entries on the elevated plus-maze in mice. Bars represents mean ± S.E.M. *P < 0.05 compared to control group.

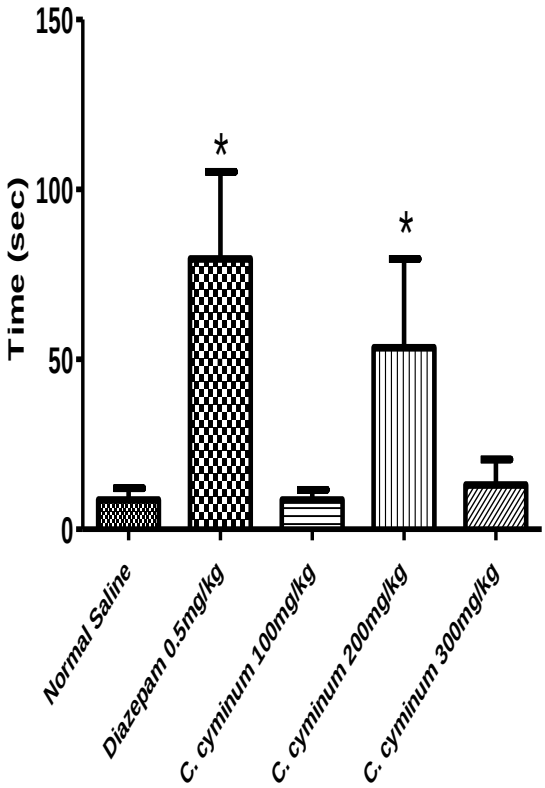

Fig 4.6. The effect of diazepam and ethanolic extract of *C. cyminum* seeds (100, 200, 300 mg/ kg) on time spent in open arm on the elevated plus-maze in mice. Bars represents mean ± S.E.M. *$P < 0.05$ compared to control group.

Effect of different doses of *Cuminum cyminum* extract on light and dark compartment test

Each mice was placed in the light compartment and observations were made for 5 min for the number of entries

and percentage of time spent in light compartment. Diazepam at dose 0.5 mg/kg significantly increase in number of entries and percentage of time spent in light compartment (*P* less than 0.05) compared to normal saline treated group. Percentage of time spent in the light compartment was significantly increased (*P* less than 0.05) with 200 mg/kg dose of C. *cyminum* extract compared to normal saline treated group. Doses 100 mg/kg, 200 mg/kg, and 300 mg/kg of extract had no significant effect on number of entries in light compartment and doses 100 mg/kg and 300 mg/kg did not have significant increase on the percentage of time spent in light compartment (fig 4.7, fig 4.8).

Table 4.2: Effect of different doses of ethanolic extract of *Cuminum cyminum* on light and dark compartment test

Groups	Treatments	Doses	Number of entries in light compartment	Percentage of time spent in light compartment
I	Normal Saline	0.1 ml/10g	4.80 ± 1.65	21.40 ± 7.63
II	Diazepam	0.5 mg/kg	(13.80 ± 2.33) *	(59.66 ± 6.78) *
III	C. cyminum extract	100 mg/kg	9.60± 1.43	42.40 ± 6.56
IV	C. cyminum extract	200 mg/kg	10.00 ± 2.5	(47.05 ± 5.47) *
V	C. cyminum extract	300 mg/kg	8.00 ± 2.77	40.13 ± 10.52

*: showing significant value, $P < 0.05$

Values are expressed as Mean ± SEM, (n=5)

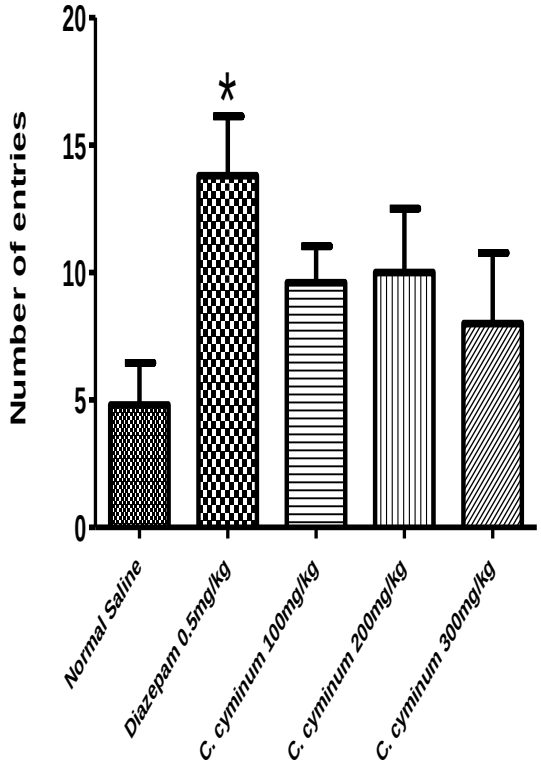

Fig 4.7. The effect of Diazepam and ethanolic extract of *C. cyminum* seeds (100, 200, 300 mg/kg) on number of entries in light compartment of light and dark compartment test. Bars represents mean ± S.E.M. **P* < 0.05 compared to control group.

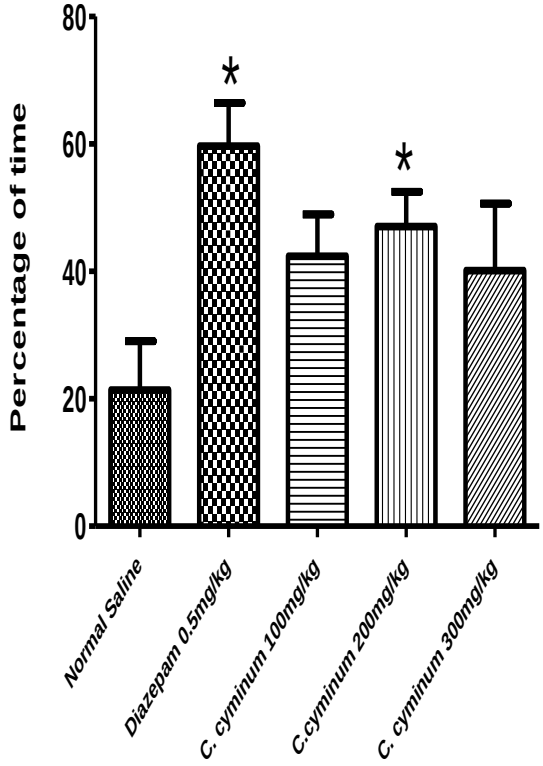

Fig 4.8 The effect of Diazepam and ethanolic extract of *C. cyminum* seeds (100, 200, 300 mg/kg) on percentage of time spent in light compartment of light and dark compartment. Bars represents mean ± S.E.M. **P* < 0.05 compared to control group.

DISCUSSION

Research on medicinal plants and use as nutritional supplements is increasing, now a days. Due to advantageous effect medicinal plants research have greater interest in medicinal plants. Many studies have done on *C. cyminum,* and its different pharmacological activities have been studied. Antispasmodic activity, appetite stimulant, antihyperglycemic activity [14], antimicrobial activity [15], high antioxidant activity [16], hypolipidemic effect [17], anticancer activity, inhibition of colonic cancer [18] and inhibition of arachidonate-induced platelet aggregation by ether extract of *C. cyminum* [19] have been studied.

In this study anxiolytic activity of ethanolic extracts of *C. cyminum* in mice has been assessed by using two models of anxiety: EPM model and light and dark compartment. Dose of 200 mg/kg of *C. cyminum* extract had significant anxiolytic action on EPM (Increase in percentage of entries and time spent in open arms) and light and dark compartment test (increase in number of entries and percentage of time spent in light compartment) in male albino mice. These observations showed that *C. cyminum* has anxiolytic activity and this activity is comparable to the Diazepam (0.5 mg/kg).

Mechanism was determined by blocking the $GABA_A$ receptor by Flumazenil (BZD site antagonist) then administered the doses of *C. cyminum* (200 mg/kg). Pretreatment with Flumazenil had marked decrease in their anxiolytic activity. This showed that anxiolytic activity of *C. cyminum* may have same mechanism as the BZD that have action through $GABA_A$ receptor because flavonoids and BZD have similar structure.

CONCLUSION

Summarizing this, the ethanolic extract of *C. cyminum* have anxiolytic activity. Biochemical investigation showed that some flavonoids are present in the extracts, and they may act through the $GABA_A$ receptor to have their anxiolytic action. More studies are required.

1. To quantify the number of flavonoids, present in the ethanolic extracts and separate

the flavonoids from the extracts to use it for further studies.

2. Investigate the exact mechanism involve in anxiolytic activity.
3. Formulate suitable dosage forms of extracts of *C. cyminum.*
4. Dosage form in combination of both can be formulated to have maximum anxiolytic activity. So that patient can use them with ease and having better results.

ACKNOWLEDGEMENT

We acknowledge technical help of central research lab, Department of Pharmaceutical Sciences of Lahore College for Women University, Lahore for their technical support.

ETHICAL APPROVAL

Ethical approval of this research study was obtained from the **Institutional Review Board** of Lahore College for Women University.

REFERENCES

1. Chorpita, B.F. and D.H. Barlow, *The development of anxiety: the role of control in the early environment.* Psychological bulletin, 1998. **124**(1): p. 3.
2. Shekhar, A., J. Hingtgen, and J. DiMicco, *GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats.* Brain research, 1990. **512**(1): p. 81-88.
3. Wasowski, C. and M. Marder, *Flavonoids as GABAA receptor ligands: the whole story?* Journal of experimental pharmacology, 2012. **4**: p. 9.
4. Girish, C., et al., *Involvement of the GABAergic system in the anxiolytic-like effect of the flavonoid ellagic acid in mice.* European Journal of Pharmacology, 2013. **710**(1-3): p. 49-58.
5. Johnston, G.A., et al., *Modulation of ionotropic GABA receptors by natural products of plant origin.* Advances in pharmacology, 2006. **54**: p. 285-316.
6. Chinnabattigalla, S., R.K. Dakoju, and S. Gedu, *Recent advances on the synthesis of flavans, isoflavans, and neoflavans.* Journal of Heterocyclic Chemistry, 2021. **58**(2): p. 415-441.
7. Nielsen, M., S. Frøkjaer, and C. Braestrup, *High affinity of the naturally-occurring biflavonoid, amentoflavon, to brain benzodiazepine receptors in vitro.* Biochemical pharmacology, 1988. **37**(17): p. 3285-3287.
8. Madhukar, C., *Phytochemical screening of cumin seeds extract. Rep. Opin., 5 (1): 57-58*. 2013.
9. Chithra, V. and S. Leelamma, *Hypolipidemic effect of coriander seeds (Coriandrum sativum): mechanism of action.* Plant Foods for Human Nutrition, 1997. **51**(2): p. 167-172.
10. Gohari, A.R. and S. Saeidnia, *A review on phytochemistry of*

Cuminum cyminum seeds and its standards from field to market. Pharmacognosy Journal, 2011. **3**(25): p. 1-5.
11. Al-Snafi, A.E., *The pharmacological activities of Cuminum cyminum-A review.* IOSR Journal of Pharmacy, 2016. **6**(6): p. 46-65.
12. Lister, R.G., *The use of a plus-maze to measure anxiety in the mouse.* Psychopharmacology, 1987. **92**(2): p. 180-185.
13. Bourin, M. and M. Hascoët, *The mouse light/dark box test.* European journal of pharmacology, 2003. **463**(1-3): p. 55-65.
14. Roman-Ramos, R., J. Flores-Saenz, and F. Alarcon-Aguilar, *Anti-hyperglycemic effect of some edible plants.* Journal of Ethnopharmacology, 1995. **48**(1): p. 25-32.
15. Iacobellis, N.S., et al., *Antibacterial activity of Cuminum cyminum L. and Carum carvi L. essential oils.* Journal of agricultural and food chemistry, 2005. **53**(1): p. 57-61.
16. El-Ghorab, A.H., et al., *A comparative study on chemical composition and antioxidant activity of ginger (Zingiber officinale) and cumin (Cuminum cyminum).* Journal of agricultural and food chemistry, 2010. **58**(14): p. 8231-8237.
17. Dhandapani, S., et al., *Hypolipidemic effect of Cuminum cyminum L. on alloxan-induced diabetic rats.* Pharmacological research, 2002. **46**(3): p. 251-255.
18. Gagandeep, et al., *Chemopreventive effects of Cuminum cyminum in chemically induced forestomach and uterine cervix tumors in murine model systems.* Nutrition and cancer, 2003. **47**(2): p. 171-180.
19. Srivastava, K., *Extracts from two frequently consumed spices—cumin (Cuminum cyminum) and turmeric (Curcuma longa)—inhibit platelet aggregation and alter eicosanoid biosynthesis in human blood platelets.* Prostaglandins, leukotrienes and essential fatty acids, 1989. **37**(1): p. 57-64.