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Anxiolytic and anticonvulsant activity of methanolic extract of allium cepa Linn (Onion) bulbs in Swiss albino mice

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Abstract
The objective of the present study was to evaluate the anxiolytic and anticonvulsant activity of the methanolic extract of *Allium Cepa Linn* (MEAC). After preliminary phytochemical evaluation, acute oral toxicity test, anxiolytic activity of methanolic extract of *Allium Cepa* bulbs at doses of 200 and 400 mg/kg was assessed using elevated-plus-maze (EPM), open field test (OFT), light & dark transition (L&DT) models and anticonvulsant effect was assessed using maximal electroshock (MES) and Isoniazid (INH) induced seizure models. Oral administration of MEAC for seven days significantly increased number of entries and time spent in open arms in EPM model; latency, number of squares crossed and time spent in Central Square in OFT; time spent in light zone and number of transitions in LDT model. Further, MEAC (200 and 400 mg/kg) showed significant reduction in the duration of hind limb extensor phase in electroshock convulsions; protected the mice against the Isoniazid induced convulsions. Mechanistic studies showed significant improvement in brain GABA levels after *Allium cepa* treatment.

Keywords: Allium Cepa, Anxiolytic, Anticonvulsant, GABA.

1. Introduction
Anxiety is an apprehension or excessive fear about real or imagined circumstances. The central characteristic of anxiety is worry or fear. Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. These seizures are transient signs and/ or symptoms of abnormal, excessive, or synchronous neuronal activity in the brain. Anxiety and epilepsy are biological disorders that affect one-eighth of the total population of the world and majority of the patients living in the developing countries, where three-fourths of the patients are not receiving adequate treatment [1, 2].

Pharmacotherapeutic approaches for the management of these ‘‘modernization-borne diseases’’ include psychotropic drugs such as barbiturates, benzodiazepines, azapirones, nor epinephrine and serotonin-reuptake inhibitors, monoamine oxidase inhibitors, and phenothiazines. Among these, benzodiazepines are the most widely prescribed synthetic chemical drugs for the treatment of anxiety, insomnia, epilepsy, and stress. Regular use of benzodiazepines causes deterioration of cognitive functioning, addiction, physical dependence, and tolerance. The standard anticonvulsant and anxiolytic drugs have a limited spectrum of activities with adverse effects that have limited their clinical usefulness and compromised patients’ compliance [3, 4]. Thus, there is need to search for newer agents with better clinical profiles for the relief of anxiety symptoms and treatment of convulsions.

Researchers of today are exploring natural resources to discover safer and cost effective drugs. Investigating plants, based on their use in traditional systems of medicine, is a sound, viable and cost-effective strategy to develop new drugs [5]. *Allium cepa* Linn. (Family; *Alliaceae*), commonly known as onion in English and other vernacular names includes Cyvannulli, Pyaz. It is a biennial (or) perennial herb with aromatic fleshy underground bulb; leaves are linear, hollow; flowers are many. *Allium cepa* is distributed throughout temperate regions of the world including Europe, Asia, North America and Africa [6]. Hence, the present study has been undertaken to elucidate the anxiolytic and anticonvulsant effect of methanolic extract *Allium cepa* Linn.

2. Materials and Methods
2.1. Plant material: The bulbs of *Allium cepa* Linn. Were procured from the local market. The plant was identified taxonomically and authenticated by Dr. B.R.C. Murthy, Department of Botany, Hindu College, Guntur.

2.2. Preparation of methanolic extract: The onion bulb was washed with freshly prepared sterile distilled water. The outer covering of the bulb was manually peeled off. Onion (*Allium cepa* Linn.) bulbs were made into fine pieces and squashed. The squashed preparation was soaked in 500 ml of methanol for 7 days with interval shaking. The extraction was filtered using muslin cloth and then Whatman no. 1 filter paper. The filtrate was evaporated at 45 ºC to dryness and the dried substance was kept in sterile bottle under refrigerated condition until use.

2.3. Preliminary phytochemical analysis: The methanolic extract of bulbs of *Allium cepa* (MEAC) was subjected to preliminary qualitative investigations [7].

2.4. Animals: Swiss albino mice of either sex weighing between 22-28g were procured from Teena labs, Hyderabad. The animals were acclimatized to laboratory conditions one week prior to experiment. The animals were fed with commercially available standard diet and water *ad libitum* under hygienic conditions. All animal studies were performed in accordance to guideline of CPCSEA with prior approval from Institutional Animal Ethical Committee (IAEC), Hindu College of Pharmacy (HCOP/IAEC/ PR-06/2014).

2.5. Acute oral toxicity: Acute toxicity studies for Methanolic extract of *Allium Cepa Linn.* Was conducted as per OECD guidelines No.423 using Swiss albino mice. The animals were administered with single dose (2000 mg/kg) of extract and observed for any changes continuously for the first 2h and up to 14 days for mortality [8].

2.6. Anxiolytic activity

A total of 24 mice were divided into 4 groups with 6 animals in each group. Group 1:- Vehicle Control (0.1% Na CMC equivalent); Group 2:- Treated with standard drug -Diazepam (2 mg/kg i.p.); Group 3:- Treated with methanolic extract *of Allium cepa* (200 mg/kg; p.o.); Group 4:- Treated with methanolic extract of *Allium cepa* (400 mg/kg; p.o.). The animals were treated with vehicle, extract 60 min and Diazepam 30 min prior to the test.

2.6.1. Elevated plus-maze model

The plus-maze apparatus consisting of two open arms (30 x 5 CM)) and two closed arms (30cm x 5cm x 15cm) extending from central platform and was elevated to a height of 50 cm above the floor. After treatment, each mouse was individually placed on the center of the elevated plus maze with its head facing the open arm. During the 5 min experiment, following behaviors of the mouse were recorded; Number of entries into open and closed arm, time spent in open & closed arms and time spent in neutral zone [9].

2.6.2. Light and dark transition model

Light-dark transition box (40cm×20cm×20cm) consists of two parts, the light-compartment and the dark compartment with a volume ratio of 3:1. After treatment, mice were placed individually in the light-dark box. During the test the mice were put into the center of the light compartment with their back to dark compartment and then transition behavior over 5 min period was observed; Latency, number of crossings between the light and dark area, total time spent in the illuminated part of the box, total rearings [10].

2.6.3. Open field test

The apparatus consists of wooden box of (68 x 68 x 45 CM) and the floor of the box was divided into 16 squares (15x15 CM). After treatment, each mouse was placed individually in the apparatus. During the 5 min experiment, following behaviors of the mouse were recorded; latency, center square duration, number of rearing, number of squares crossed [11].

2.7. Anticonvulsant activity

A total of 24 mice were divided into 4 groups, each group containing 6 animals. Group 1:- Vehicle control (0.1% Na CMC equivalent) Group 2:- Treated with standard drugs - Phenytoin (5 mg/kg; i.p.) in maximal electroshock induced convulsion model and Diazepam (5 mg/kg; i.p.) in Isoniazid induced convulsion model. Group 3:- Treated with methanolic extract *of Allium cepa* (200 mg/kg; p.o.) Group 4:- Treated with methanolic extract of *Allium cepa* (400 mg/kg; p.o.). The animals were treated with vehicle, extract 60 min; Phenytoin and Diazepam 30 min prior to the test.

2.7.1. Maximal electroshock induced convulsions (MES)

After treatment, an electric shock (50 mA for 0.2 sec) using an electro convulsiometer was applied through the corneal electrodes. Mice were placed in a rectangular plastic cage with an open top, permitting full view of animal's motor responses to seizure and different phases for each animal. The severity of convulsions was assessed by the duration of flexion, extension, clonus, stupor and recovery phase for each animal [12].

2.7.2. Isoniazid induced convulsions (INH)

It is comes under chemically induced convulsions. After treatment, INH (Isoniazid 300 mg/kg) was administered by oral route to all the animals. Each animal was placed into individual plastic cage and were observed initially for 30 min and later up to 24h.The following parameters were recorded during test session; Latency to seizures and status of the animal [13].

2.8. Quantitative estimation of GABA levels in brain tissue

In this method, 1.0 ml of the supernatant from brain homogenate was evaporated to dryness at 70 °C in an oven. Standard solutions of GABA at a concentration of 2mM along with the sample are spotted on Whatman No. 1 chromatography paper using a micropipette. When the solvent front reached the top of the paper, it were removed and dried. A second run were performed similarly, after which the papers are dried sprayed with ninhydrin reagent and placed in an oven at 100 °C for 4 minutes. The portions which carry GABA corresponding with the standard are cut and eluted with 0.005% $CuSo_4$ in 75% ethanol. Their absorbencies were read against the blank at 515 nm in spectrophotometer. Standard graph was prepared with GABA and the values were expressed as mcg/ml of GABA/g wet weight [14].

3. Results

3.1. Preliminary phytochemical analysis

Preliminary phytochemical analysis of methanolic extract of *Allium cepa* showed the presence of carbohydrates, flavonoids, saponins, glycosides, tannins and polyphenols.

3.2. Acute oral toxicity

There was no mortality and noticeable behavioral changes in all the groups tested. The extract was found to be safe upto a dose level of 2000 mg/kg body weight.

3.3. Anxiolytic activity

After treatment with MEAC in mice there was significant increase in the number of open arm entries as well as time spent in the open arm at dose levels of 200 mg/kg and 400 mg/kg of MEAC when compared to control in Elevated plus maze (EPM) test (Table 1). Further, significant increase in the number of crossings as well as time spent in light zone in Light and dark transition (L&DT) model (Table 2). In open field test (OFT), significant reduction in latency time and increase in number of squares crossed, number of rearings as well as time spent in Central Square (Table 3). Moreover, the anxiolytic effect of the MEAC at high dose (400 mg/kg) was comparable to that of Diazepam.

Table 1: Effect of methanolic extract of *Allium cepa* bulbs in elevated plus maze (EPM) model

Treatment	Time spent in arms (sec)		Number of entries into arms		Time spent in neutral zone (sec)
	open arm	closed arm	open arm	closed arm	
Control (0.1% Na CMC equivalent)	25.5±3.11	207.3±16.5	3.1±0.57	9.0±0.96	48.3±0.33
Standard (Diazepam; 2 mg/kg, i.p.)	149.17±7.09***	93.0±0.96***	14.5±1.02***	5.32±0.95**	45.1±0.16
Test Group- I (MEAC ; 200 mg/kg p.o)	83.72±10.96***	142.8±3.10**	7.57±0.63*	7.8±0.98	78.5±0.34
Test Group-II (MEAC ; 400 mg/kg p.o)	124.24±5.21***	108.2±3.18***	10.8±1.07***	5.6±1.02*	84.3±0.21*

Values were expressed as mean ± SEM (n=6). Values were statistically significant at *P<0.05, **P<0.01, ***P<0.001 Vs control using one way ANOVA followed by Dunnett's't' test.

Table 2: Effect of methanolic extract of *Allium Cepa* bulbs in light and dark transition (L&DT) model

Treatment	Transfer latency (sec)	Time spent in light zone (sec)	Number of crossings in to light zone
Control (0.1% Na CMC equivalent)	4.32±0.52	97.6±0.88	6.3±0.66
Standard (Diazepam; 2 mg/kg, i.p.)	18.87±2.12***	154.0±0.57***	21.0±0.96***
Test Group I (MEAC; 200 mg/kg, p.o.)	10.28±1.89*	135.0±0.96**	11.5±0.34*
Test Group-II (MEAC; 400 mg/kg, p.o.)	16.33±3.34**	152.2±0.40***	16.1±0.30***

Values were expressed as mean ± SEM (n=6). Values were statistically significant at **P<0.01, ***P<0.001 Vs control using one way ANOVA followed by Dunnett's't' test.

Table 3: Effect of methanolic extract of *Allium cepa* bulbs in open field test (OFT) model

Treatment	Latency (sec)	Number of squares crossed	Time spent in central square (sec)	Number of rearing
Control (0.1% Na CMC equivalent)	79.3±5.21	17.7±0.42	32.8±0.64	10.3±1.61
Standard (Diazepam; 2 mg/kg, i.p.)	8.19±1.30***	68.0±0.89***	205.0±0.63***	28.1±3.47***
Test Group I (MEAC; 200 mg/kg, p.o)	28.3±4.42 ***	32.5±0.76**	95.0±0.84**	18.27±1.79*
Test Group-II (MEAC ; 400 mg/kg, p.o)	17.5±2.34***	54.2±0.54***	156.7±0.60***	25.3±2.42***

Values were expressed as mean ± SEM (n=6). Values were statistically significant at **P<0.01, ***P<0.001 Vs control using one way ANOVA followed by Dunnett's't' test.

3.4. Antiepileptic activity

After treatment with MEAC at dose level of 200 and 400 mg/kg, significant reduction in the duration of hind limb extensor phase and overall reduction in the duration of convulsive period in case of maximal electroshock induced convulsions test (Table 4). In Isoniazid induced convulsions, treatment with MEAC (200 and 400 mg/kg) significantly delayed the onset of seizures and protected the mice when compared to control (Table 5).

Table 4: Effect of methanolic extract of *Allium cepa* in maximal electroshock (MES) induced convulsion model.

Treatment	Flexion (sec)	Extension (sec)	Clonus (sec)	Stupor (sec)
Control (0.1% Na CMC equivalent)	6.6±0.66	13.8±0.60	15.0±0.73	86.81±6.60
Standard (Phenytoin; 5mg/kg, i.p.)	2.3±0.76***	0.00***	7.8±0.47***	28.42±2.51***
Test Group I (MEAC ; 200 mg/kg p.o)	5.1±0.42	4.5±0.34**	12.0±0.77	55.17±5.79*
Test Group-II (MEAC ; 400 mg/kg p.o)	3.8±0.54**	1.6±0.42***	10.8±0.79*	39.14±4.40**

Values were expressed as mean ± SEM (n=6). Values were statistically significant at **P<0.01, ***P<0.001 Vs control using one way ANOVA followed by Dunnett's't' test.

Table 5. Effect of methanolic extract of *Allium cepa* on Isoniazid (INH) induced convulsions in mice

Treatment	Latency of seizures (Sec)	No. convulsed/No. mice	Protection from death (%)
Control (0.1% Na CMC equivalent + Isoniazid; 300 mg/kg p.o)	54±0.17	6/6	0
Standard (Diazepam; 5 mg/kg, i.p.)	A	0/6	100
Test Group –I (MEAC; 200 mg/kg, p.o)	76.12±5.17**	5/6	33.33
Test Group-II (MEAC; 400 mg/kg, p.o)	109.33±7.11***	2/6	83.33

A= absence of convulsion. Values were expressed as mean ± SEM (n=6). Values were statistically significant at **P<0.01, ***P<0.001 Vs control using one way ANOVA followed Dunnett's't' test.

3.5. Quantitative estimation of GABA levels in whole brain of mice

After 7 days of treatment of mice with MEAC, there was significant increase in whole brain GABA levels at dose levels of 200 mg/kg and 400 mg/kg of MEAC when compared to Isoniazid (INH) control mice (Table 6).

Table 6: Quantitative estimation of GABA in whole brain of mice

Group	Treatment	Brain GABA (ng/gm)
I	Control (0.1%Na CMC equivalent)	3008.26±31.17
II	Negative control (Isoniazid; 300mg/kg, p.o.)	1599.01±42.01$^{\$\$}$
III	Test Group I (MEAC; 200 mg/kg, p.o.)	2127.19±58.80*
IV	Test Group-II (MEAC; 400 mg/kg, p.o.)	2533.71±42.06**

$$-P<0.01 – Negative control compared with normal control mice, *P<0.05, **P<0.01, Individual readings were compared with readings of negative control using One way ANOVA followed by Dunnett's 't' test (n = 6).

4. Discussion

In the present study, anxiolytic activity of *Allium cepa* was evaluated by three different screening models. Elevated plus maze test is used to evaluate psychomotor performance and emotional aspects of rodents [15]. Light/dark test is another widely used animal model of anxiety and it is based on the on the spontaneous exploratory behaviors of rodents in response to novel environments and light [16]. The open field model examines anxiety-related behavior characterized by the normal aversion of the animal to an open, brightly lit area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters [17]. MEAC at dose level of 200 and 400 mg/kg showed significant increase in time spent as well as number of entries into open arms when compared to control as in case of elevated plus maze test, significant increase in the number of crossings as well as time spent in light zone when compared to control as in case of light and dark test and significant increase in number of squares crossed, number of rearings as well as time spent in central square and latency time when compared to control as in case of open field test, suggesting the anxiolytic activity of methanolic extract of *Allium cepa*. Moreover, the anxiolytic effect of the MEAC at 400 mg/kg was comparable to that of standard drug diazepam.

In the present study, anticonvulsant activity was evaluated using two different screening models. Maximal electroshock induced convulsions test is assumed to identify the antiepileptic drugs effective against generalized tonic-clonic seizures and Isoniazid induced convulsions test is assumed to identify the antiepileptic drugs effective against absence seizures [18]. In MES induced convulsions model, repetitive administration of exogenous high-frequency electric stimulations leading to uncoordinated and excessive neuronal firing and development of convulsions [19]. MEAC at dose level of 200 and 400 mg/kg showed significant reduction in the duration of hind limb extensor phase in case of maximal electroshock induced convulsions. INH induces seizures by interfering with GABA synthesis through inhibition of glutamic acid decarboxylase (GAD) activity, leading to rapid depletion of GABA [20]. In our present study, treatment with *Allium cepa* significantly delayed the onset of seizures and protected the animals from INH induced seizures.

GABA appears to play an important role in the pathogenesis of several neuropsychiatric disorders. Further, studies have proved that the agents which increase the brain GABA content and administration of centrally active GABA mimetic agents have been used as effective therapeutic approach for treatment of anxiety and epilepsy [21]. Many of the traditional agents used to treat psychiatric disorders are known to act, at least in part, by enhancing GABA activity, while some of the newer agents may exert their therapeutic effects exclusively through GABA ergic actions [22]. In our present investigation, treatment with MEAC at dose levels of 200 and 400 mg/kg showed significant increase in whole brain levels of GABA when compared to control. Flavonoids are known as positive modulators of $GABA_A$ receptors at low dose [22]. Hence, presence of flavonoids in *Allium cepa* could be responsible partly for its anxiolytic and antiepileptic action through GABA modulation.

5. Conclusion

The results of this study show that the methanol extracts of bulbs of *Allium cepa* possess anxiolytic and anticonvulsant properties which are possibly mediated partly via facilitation of GABA transmission. These results suggest that the bulbs of *Allium cepa* will be beneficial in the management of anxiety and seizures. Further studies on the isolation of the active constituents and exact mechanism of action are needed.

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