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SEDATIVE AND HYPNOTIC ACTIVITY OF BULBS OF *ALLIUM CEPA* LINN.

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ABSTRACT: The onion (*Allium cepa* L), also known as the bulb onion or common onion, is used as a vegetable and is the most widely cultivated species of the genus *Allium*. *Allium cepa* L belongs to the family Lilliaceae. The onion has a wide range of beneficial actions on the body and when eaten (especially raw) on a regular basis will promote the general health of the body. The parameters selected were preliminary phytochemical screening and sedative hypnotic activity by chimney test. The *Allium cepa* Linn powder was successively extracted with benzene, chloroform, ethylacetate, ethanol and water for the identification of the best solvent. Preliminary phytochemical screening was carried out for all the extracts and maximum chemical constituents were observed in the ethanolic extract. The sedative and hypnotic activity were tested by using ethanolic extract on mice by using chimney test and found to be effective at a dose of mg/kg when compared to the reference standard. In conclusion it can be said that *Allium cepa* L possess Sedative and hypnotic activity at a dose of mg/kg.

INTRODUCTION: A sedative or hypnotic drug is one that reversibly depresses the activity of the central nervous system, used chiefly to induce sleep and to allay anxiety. Recent studies have shown that herbal drugs exert good sedative and hypnotic effect on the central nervous system [1-3]. The scientific literature has demonstrated that different extracts and compounds of onion produce significant biological effects [4]. Onion (*Allium cepa*) bulb from Liliaceae has antioxidant, spasmolytic and antihypertensive activities. Calcium channels were involved in the onion peel hydroalcoholic extract spasmolytic effect on rat ileum. Flavonoids extracted from onion peel improve male sexual function [5, 6].

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Natural antioxidants allowing for the substitution of synthetic antioxidants are the target of many studies. Boo et al demonstrated high antioxidant activities of natural pigments found in onion [7, 8]. Onion (*Allium cepa*) varieties are rich in quercetin (flavonol). Their flavonoids content and antioxidant activity was evaluated using MAE as the extraction techniques [9].

The aim of this experiment is to evaluate the sedative and hypnotic activities of *Allium cepa* L. ethanolic and therefore to determine the scientific basis for its use in traditional medicine in the management of central nervous system disorders.

MATERIALS AND METHODS: The plant was collected from local market of Bhongir. It was identified and authenticated by Prof. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in

the Dept. of Pharmacognosy for further reference. The plant was identified as *Allium cepa* L. (Lilliaceae) under the voucher no: SSCP/2012-2013-001.

Instruments and Chemicals used: The solvents used for extraction were, Benzene, Chloroform, Ethyl acetate, Ethanol and Distilled Water. Other reagents used were of laboratory grade and obtained from various other commercial sources. All the reagents used were of laboratory and analytical grade. Solvents are obtained from SD Fine-Chem Ltd.(Mumbai), Virat Lab company (Hyderabad), Accord labs (Secunderabad), Rolex laboratory reagent (Mumbai), Nova Biotech (Kolkata), Diazepam tablets (Barr Laboratories)

Pharmacognostic studies:

Extraction: The collected aerial parts of the plant were washed and dried under the shade. Around 25 g of the coarsely powdered aerial parts of the plant was packed in a Soxhlet apparatus and exhaustively extracted with the solvents of increasing polarity. The extract so obtained was concentrated under vacuum using rotary vacuum evaporator and dried in desiccator until use.

Preliminary Phytochemical Screening [10, 11, 12-16]: The extract so obtained were subjected to various chemical tests as per the procedure mentioned in the standard reference books to determine the nature of chemical constituents present the in the plant .

Animals: Albino mice of either sex weighing 20-40gm were used for animal studies .The animals were grouped in Polyacrylic cages and maintained under standard laboratory conditions (Temp 27 ± 2 °C) and relative humidity (50 ± 5%)with dark and light cycle (14/10 hrs).They were allowed for free access to standard dry pellet diet and water *ad libitum.*

The mice were acclimatized to laboratory conditions for 10 days before commencement of experiment. The experiment was carried out according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethical Committee (IAEC) approved all procedures.

The Acute toxicity test was done according to OECD guideline – 423, selected with three male and three female for 7-14 days.

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Method to test Sedative and Hypnotic activity: The investigation of hypnotic and sedative effect of *Allium cepa* is carried out by chimney test in mice and grip strength in mice.

Chimney test in mice:

- **Procedure** – Male mice weighing between 16 to 22 are used. Pyrex-glass cylinders 30 cm long are required. The internal diameter varies with the animal's weight: for mice weighing 16 to 18 g, the diameter is 22 mm, for mice weighing 18 to 20 g, 25 mm; for mice weighing 20 to 22 g, 28 mm. Each tube has a mark 20 cm from its base. Initially, the tube is held in a horizontal position. At the end of the tube, near the mark, a mouse is introduced with the head forward. When the mouse reaches the other end of the tube, toward which it is pushed if necessary with a rod, the tube is moved to a vertical position.

  Immediately, the mouse tries to climb backwards and performs coordinated movements similar to an alpinist to pass a chimney in the mountains. The time required by the mouse to climb backwards out at the top of the cylinder is noted. The ED50with 95%confidence limits i.e. the dose at which 505of the animals fail to climb backwards within 30 sec is calculated using log probit analysis method [17].

Grip strength in mice: The test is being used to assess muscular strength or neuromuscular function.

- **Procedure** - Male or female mice with an average weight of 22 g are used. The animals are exposed to a horizontal thin threat or metallic wire suspended about 30 cm into the air which they immediately grasp with the forepaws. The mouse is released to hang on with its forelimbs. Normal animals are able to catch the threat with the hind limbs and to climb up within 5 s. After oral or subcutaneous administration the animals are tested every 15 min. Animals which are not able to touch the threat with the hind limbs within 5 s or fall off from the threat are considered to be impaired by drug effect.

  After the completion of this test the animals are observed for their behavior and mobility in the cages. If their behavior and mobility in the cage appearance to be normal. The disturbance of the grasping reflux can be considered to be caused by central relaxtion the percentage of animals losing the grip strength is recorded using different dose of test or standard drugs and ED50 values are calculated. The response curve can also be plotted using the data of these experiments [18].

RESULTS AND DISCUSSIONS:

FIGURE 1: PHOTOGRAPH OF BULB OF *ALLIUM CEPA* L.

Extraction:

- Extraction was carried out by the Soxhletion based on the increasing order of polarity as follows:

Nature and % yield of different extracts:

TABLE 1:

Extracts	Color	Consistency	%w/w
Benzene	Brown	Oily	1.775
Chloroform	Brown	Oily	2.5
Ethyl acetate	Brown	Gummy	1.65
Ethanol	Brown	Oily	4
Water	Brown	Gummy	14.8

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE VARIOUS AERIAL PARTS EXTRACTS OF *ALLIM CEPA* L:

Test	Benzene	Chloroform	Ethyl acetate	Ethanol	Water
Carbohydrates	-	-	-	-	+
Proteins	-	-	-	+	-
Amino acids	-	-	-	+	-
Fats and oils	-	-	-	+	-
Steroids	-	-	-	-	-
Glycosides	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Flavonoids	-	+	+	+	-
Alkaloids	+	-	-	-	+
Saponins	+	+	-	+	-
Tannins	+	+	+	+	+

Acute toxicity test:

Acute Toxicity of Ethanolic extract of *Allium cepa* L: Following oral administration *Allium cepa* L. extract at the doses of 500, 1000, 1500, 2000mg/kg, p.o., no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at these doses. This result indicates that, the LD50 was higher than 2000 mg/kg.

Chimney test in mice: In control group, the sedation was maximum at 90min as recorded in **Table 3**.

In standard group (Diazepam) has shown significant sedative and hypnotic activity and p-value using ANOVA were recorded in Table 3.

In test group, low dose (300mg/kg) the sedation was maximum at 30min after which the paw volume decrease gradually and therefore readings were recorded in Table 3.

In test group, high dose (500mg/kg) the sedation was maximum at 30min after which the paw volume decreases gradually and therefore readings were recorded in Table 3.

The standard deviation (SD), Standard error mean(SEM)was calculated between control & all other groups Table 3.

TABLE 3: HYPNOTIC AND SEDATIVE ACTIVITY OF EEAC USING CHIMNEY TEST IN MICE METHOD

S. No.	Treatment	Dose (ml/kg/ml)	Mean difference in Sedation (ml.) ± S.E.M		
			30min	60min	90min
1	Control	-	1.3±0.2	1.2±0.2	1.5±0.22
2	Standard	0.24	6.0±0.25**	4.83±0.40	4.6±0.49
3	Test(low)	200mg/Kg	7.16±0.4***	4.66±0.49	3±0.36
4	Test(high)	400mg/Kg	7.3±0.33***	6.0±0.36	3.83±0.6

***=p-value<0.001, **=p-value<0.01 done by stastical analysis of ANOVA followed by Tukey's multiple comparision test. EEAC=Ethanolic extract of *Allium cepa* L.

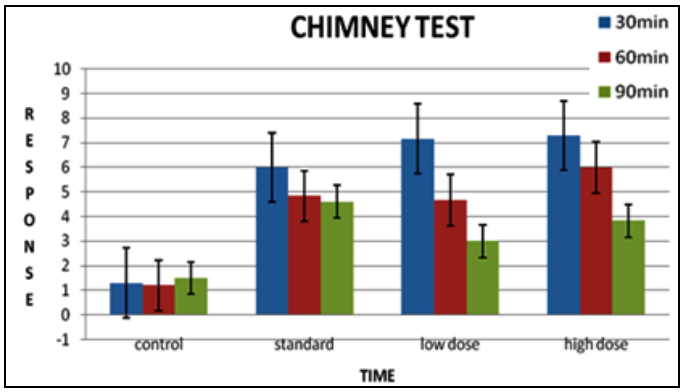

FIGURE 2: EFFECT OF EEAC AT VARIOUS TIME INTERVALS USING CHIMNEY TEST IN MICE METHOD

Grip strength in mice: In control group, the sedation was maximum at 90min as recorded in **Table 4**.

In standard group (Diazepam) has shown significant sedative and hypnotic activity and p-value using ANOVA were recorded in Table 4.

In test group, low dose (300mg/kg) the sedation was maximum at 60min after which the paw volume decrease gradually and therefore readings were recorded in Table 4.

In test group, high dose (500mg/kg) the sedation was maximum at 60min after which the paw volume decreases gradually and therefore readings were recorded in Table 4.

The standard deviation (SD), Standard error mean (SEM) was calculated between control & all other groups Table 4.

TABLE 4: HYPNOTIC AND SEDATIVE ACTIVITY OF EEAC USING GRIP STRENGTH IN MICE METHOD

S. No.	Treatment	Dose (ml/kg/ml)	Mean difference in Sedation (ml.) ± S.E.M		
			30min	60min	90min
1	Control	-	5.3±0.3	5.5±0.34	6.5±0.34
2	Standard	0.24	5±1.8	14.16±0.65**	9.3±0.42
3	Test(low)	300mg/kg	11.16±0.6	12.16±0.54*	8±0.77
4	Test(high)	500mg/kg	2.8±0.6	20.5±0.61***	6.83±0.40

***=p-value<0.001, **=p-value<0.01, *=p-value<0.005 done by stastical analysis of ANOVA followed by Tukey's multiple comparison test. EEAC=Ethanolic extract of *Allium cepa* L.

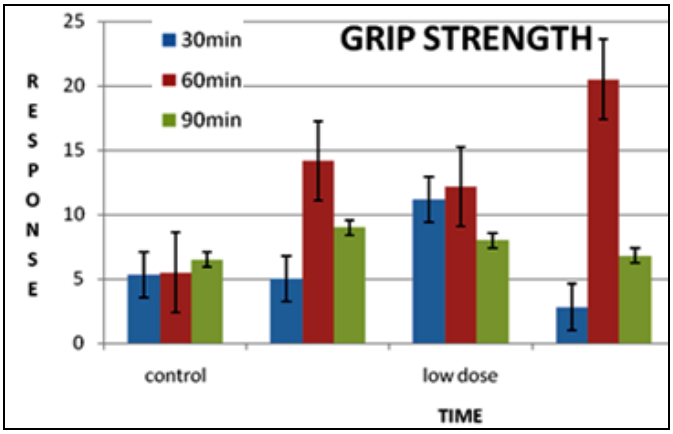

FIGURE 2: EFFECT OF EEAC AT VARIOUS TIME INTERVALS USING GRIP STRENGTH IN MICE METHOD

DISCUSSION: Diazepam is central nervous system depressant used in the management of sleep disorders such as insomnia; these compounds have a binding site on GABA receptor type-A ionophore complex ($GABA_A$) [4, 5]. It decreases activity, moderates excitement, and calms the recipient. Substances like diazepam (which has been chosen as the standard reference drug in this study) reduce onset of and increase duration of barbiturate-induced sleep and reduce exploratory activity possessing potentials as sedative [18, 19].

Diazepam is a very well-known anxiolytic benzodiazepine (BDS) which produces not only anxiolytic-like effect but also important sedative action. In this respect, *Allium cepa* L causes sedative activity by decreasing the grip strength greater than diazepam (Tables 3 and 4). It is generally believed that locomotor activity results from brain activation, which is manifested as an excitation of central neurons involving different neurochemical mechanism and an increase in cerebral metabolism. It is possible that the sedative activity of ethanolic extract of Allim cepa L is mediated by GABAergic pathway, since GABAergic transmission can produce profound sedation in mice [20].

The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnosis activity. Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain.

Preliminary phytochemical studies revealed that it contain flavonoids & saponins which might be responsible for SEDATIVE AND HYPNOTIC of EEAC decreased grip strength activities and reduced different behavioral reflexes. Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the sedative and hypnotic effect. In conclusion, p.o. administration of methanolic and aqueous extract of EEAC induces similar sedative effects, supporting its use in folk medicine.

Given that the LD_{50} value for these extracts was beyond 2000mg/kg for oral administration, as determined by Litchfield and Wilcoxon [21]; our results suggest a remote risk of acute toxicity and good tolerance of these extracts in traditional medicine. To sum up, this work represents that the Ethanolic and aqueous Allium cepa L have obvious sedative and hypnotic activity; these data provide pharmacological basis for its therapeutic efficacy on insomnia.

CONCLUSION: The present study showed that there exists a significant sedative and activity of *Allium cepa* L for 500mg/kg by grip strength and chimney test in mice. It can be concluded from the present discussion that the ethanol extract of *Allium cepa* exhibited strong SEDATIVE AND HYPNOTIC activity.The hypnotic and sedative activity of *Allium cepa* L. may be due to flavonoids and saponins. The future work may be done on investigating the confirmation of the said chemical constituents viz.., flavonoids and saponins.

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