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THE CURRENT STUDY AIMED AT INVESTIGATION OF EFFECTIVENESS OF ETHANOLIC AND AQUEOUS EXTRACT OF *HELIANTHUS ANNUS IN* ANXIOLYTIC DISESE IN EXPERIMENAL ABLINO RATS USING ELEVATED PLUS MAZE APPARATUS

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ABSTRACT:

The present study is aimed to evaluate the anxiolytic activity of ethanol and aqueous extract of ***HELIANTHUS ANNUS*** using elevated plus maze test (epm) model in rats. ethanol and aqueous extract of ***HELIANTHUS ANNUS*** had increased number of entries and time spent in open arms while they decreased the number of entries and duration of time spent in closed arm of the EPM. In a similar fashion, the diazepam increased the percentage of time spent and percentage of arm entries in the open arms (**P* <0.05, ***P* <0.01). the bark had anxiolytic activity (**P* <0.05, ***P* <0.01) in EPM.

Keywords: ***HELIANTHUS ANNUS*** ,Anxiolytic activity, Elevated plus maze test etc.

INTRODUCTION

ANXIOLYTICS

An **anxiolytic** (also **antipanic** or **anti-anxiety agent**) is a medication or other intervention that reduces anxiety. This effect is in contrast to anxiogenic agents which increase anxiety. Anxiolytic medications are used for the the treatment of anxiety disorders and their related psychological and physical symptoms.

ANXIETY

Anxiety is a naturally-occurring emotion and an innate response of the body to the environmental stimuli. Mild to moderate anxiety would increase level of performance. However, when anxiety levels exceed the tolerability of a person, anxiety disorders may occur. People with anxiety disorders can exhibit fear responses such as defensive behaviors, high levels of alertness and negative emotions, without external stimuli which induce anxiety within an individual. Those with anxiety disorders are also often found to have concurrent psychological disorders, most commonly depression. Anxiety disorders are divided into 6 types in clinical recognition. (1)

PLANT PROFILE

- The **sunflower seed** is the seed of the sunflower (*Helianthus annuus*). There are three types of commonly used sunflower seeds: linoleic (most common), high oleic and sunflower oil seeds. Each variety has its own unique levels of monounsaturated, saturated, and polyunsaturated fats. The information in this article refers mainly to the linoleic variety.(2)

Biological soure:

Sunflower is the common name for any of the plants of the genus *Helianthus* of the flowering plant family *Asteraceae* (known as the aster, daisy, or sunflower family). It also commonly is used specifically in reference to the annual plant *Helianthus annuus*, the common sunflower, which is characterized by a long stem and a large flowering head (inflorescence) with large seeds. The term sunflower also refers to this plant's seed-like fruit (commonly but incorrectly called the seeds) or the encased, edible, true seeds.

Fig 1: ***HELIANTHUS ANNUS***

Chemical constituents

Sunflower seed contains 35–42% oil and is naturally rich in linoleic acid (55–70%) and consequently poor in oleic acid (20–25%).. Research shows that sunflower oil may reduce both total cholesterol and low-density lipoprotein (LDL) cholesterol and offer antioxidant properties.

MATERIALS AND METHODS

Collection of Plant Material:

The seeds of *Helianthus Annus* L were collected in the month of Dec – jan from the coastal region of Andhra Pradesh.

Identification and Authentication:

The collected plant part (seeds) of *Helianthus Annus* L were identified and authenticated by Dr.Sathyanarayana Raju (M.Sc.,M.Phil.,Ph.D.), plant taxonomist, Department of Botany and Microbiology ,Acharya Nagarjuna University, Nagarjuna Sagar Guntur-522510,A.P.

EXTRACTION OF PLANT MATERIAL

 Drying: The collected leaves were dried for 14 days at room temperature (27-37 ^{o}C). The shade drying was done to protect, the thermo-labile phytoconstituents, if any.

 Sieving: The shade dried leaves were coarsely powdered mechanically using commercial electrical stainless steel blender, and the powdered material was passed through sieve no. 20 to remove excessive mucilaginous hair and to obtain the fine powdered drug material.

 Soxhlation: The dried powdered plant material was extracted with solvents at 60 ^{o}C for 24 hours, using soxhlet apparatus. The extracts were then filtered and dried under vacuum. The extraction process was carried out with aqueous (70%). The collected extracts were termed as aqueous extract of *Helianthus Annus* L For further study the extracts were dissolved in double distilled water for further In Vitro assays.

Procurement and Housing of Animals:

Animals were purchased from the CPCSEA registered breeder, Raghavendra Enterprises, Bangalore. The animals were housed in polyacrylic cages (38X23X10 cm) with not more than four animals per cage. The animals were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12 h light / 12 h dark cycle) and maintained humidity 60 ± 5% and an ambient temperature of 25 ± 2^{o}C. The animals were allowed to free access to standard diet and water ad libitum and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22 % Protein, 4% Fat, 4% Fiber, 36% Carbohydrates and 10% Ash w/w, supplied by Raghavendra Enterprises, Bangalore were used.

Acute toxicity studies:

Acute toxicity studies of leave extracts were studied in female mice according to the guidelines for organization of economic cooperation and development (OECD 423). According to the guidelines, the female mice were used for the test. The animals were given the proper diet and kept in 12 hours light and 12 hours dark cycle. Now the mice were kept on over-night fasting before conducting the experiment. Extracts were administered to the animals at different doses i.e. 5, 50, 500, 2000, mg/kg body weight. Now the mortality and the toxicity sign were observed continuously for 1 hour and then for 24 hours after administration of extracts ***(OECD guidelines, 2006).***

Animals:

Male albino rats weighing 150-200g of were used for the study. The animals were housed in solid- bottomed polypropylene cages and acclimatized to animal conditions. The rats were fed with commercial rats diet and water adlibation. The experiments were designed and conducted in accordance with ethical forms approved by Committee for the purpose of control and supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (ICEA)

Drug treatment:

For aqueous

a) **Elevated pluz maz apparatus**

For aqueous extract

Albino Rats into four groups of 4 animals each.

Group I – Control (2% saline)

Group II – Standard drug(diazepam- 5mg/kg i.p)

Group III – AEHA (200mg/kg p.o)

Group IV – AEHA (400mg/kg p.o)

For Ethanol extract

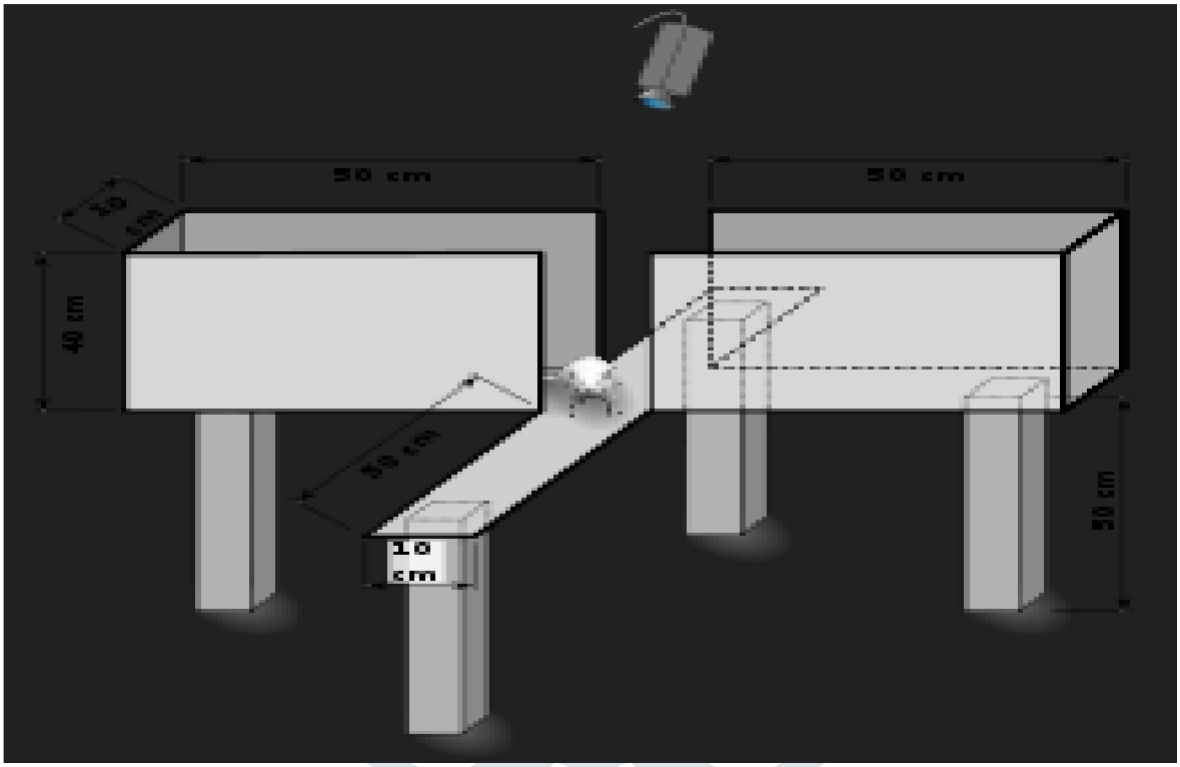
Group I – Control (2% saline)

Group II – Standard drug(diazepam- 5mg/kg i.p)

Group III – EEHA (200mg/kg p.o)

Group IV – EEHA (400mg/kg p.o)

1) Elevated plus maze method:Instrument description:



Figure;4 Elevated plus-maze test :

- The elevated plus-maze comprised two open (30 cm×5 cm×0.25 cm) and two enclosed (30 cm×5 cm×15 cm) arms that radiated from a central platform (5 cm×5 cm) to form a plus sign. The maze was constructed of black painted wood.
- A slight raised edge on the open arms (0.25 cm) provided additional grip for the animals.
- The plus-maze was elevated to a height of 40 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h).
- The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 5 min test period were used as indices of anxiety.

A mouse was considered to have entered an arm when all four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels.

STATISTICAL ANALYSIS

Results are expressed as mean ± standard error of the mean (S.E.M.). All data are subjected to analysis of variance (ANOVA) followed by Dunnetís ìtî test. P values <0.05(95% confidence limit) was considered statistically significant.

RESULTS AND DISCUSSION:

Results:

Extraction:

Size reduced powder of seeds of helianthus seeds *l* were extracted separately by Soxhlet extraction technique with aqueous (70%). Extractive yield from respective solvents.

Percentage yield of the extracts:

The percentage yield of the collected extracts was calculated accordingly and was found as mentioned in **table no.**

$$\text{Percentage yield} = \frac{\text{Weight Of extracts Obtained}}{\text{Weight of crude extracts}} \times 100$$

Table 1: Percentage yield of the collected extracts

S.NO	EXTRACT	Weight taken(grams)	Percentage yield
1	Aqueous extract of ***helianthus annus l***	200	34%
2	Ethanolic extract of ***helianthus annus l***	400	46%

Result of Phytochemical Screening:

Table 2: Result of Preliminary phytochemical screening of various extract of helianthus annus seeds

Phytochemical	Aqueous extract of *Helianthus Annus l*	Ethanol extract of *Helianthus Annus*
Carbohydrates	+	+
Glycosides	+	-
Flavonoids	-	+
Saponins	+	–
Alkaloids	+	+
Proteins and amino acids	-	–
Phenol and phenolic compounds	-	–
Tannins	-	+

'+' indicates presence'_' indicates absent

Toxicity study:

In the current exploration, the Aqueous extracts of *helianthus seeds l* were levied for studies of acute toxicity. For the determination of LD_{50} dose, Methanol extract of *helianthus seeds l* was given up to dose o f 2 gm/kg b.w. and extracts did not exhibited any sort of mortality, that's why $1/5^{th}$(400mg),$1/10^{th}$(200mg) of most dose given were preferred for thecurrent investigation .

Experiment part:

Effect of AEMC on Elevated plus maze:

In EPM saline treated animals the time spent & entries in the open and closed arms, were compared with AEMC

extract at the dose of 200mg/kg, 400mg/kg. Diazepam (5mg/kg) showed significant ($p<0.001$) increase in the time spent in the open arms and significant ($p<0.05$) increase in number of entries in open arm. Furthermore, AEMC 200, 400 mg/kg had decrease in time spent and number of entries in closed arm as Diazepam showed a significant ($p<0.05$) in elevated plus-maze.

Table No.1: Effect of AEMC on EPM paradigm in mic

Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in min(mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	**Control**	**Saline**	7.5± 0.4014	10.1± 0.9098	36.17± 0.9098	192.0 ± 3.416
II	**Diazepam**	**4mg/kg**	12.6± 0.5627***	6.5± 0.4216***	80.83±0.98042***	129.2± 2.301***
III	**AEHA**	**200**	8.7± 0.3416	9.7± 0.4014	46.33 ± 1.256**	160.2 ± 2.414***
IV	**AEHA**	**400**	10± 0.3416**	7.8± 0.3651***	62.33± 1.994***	147.00±1.713***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnettís test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

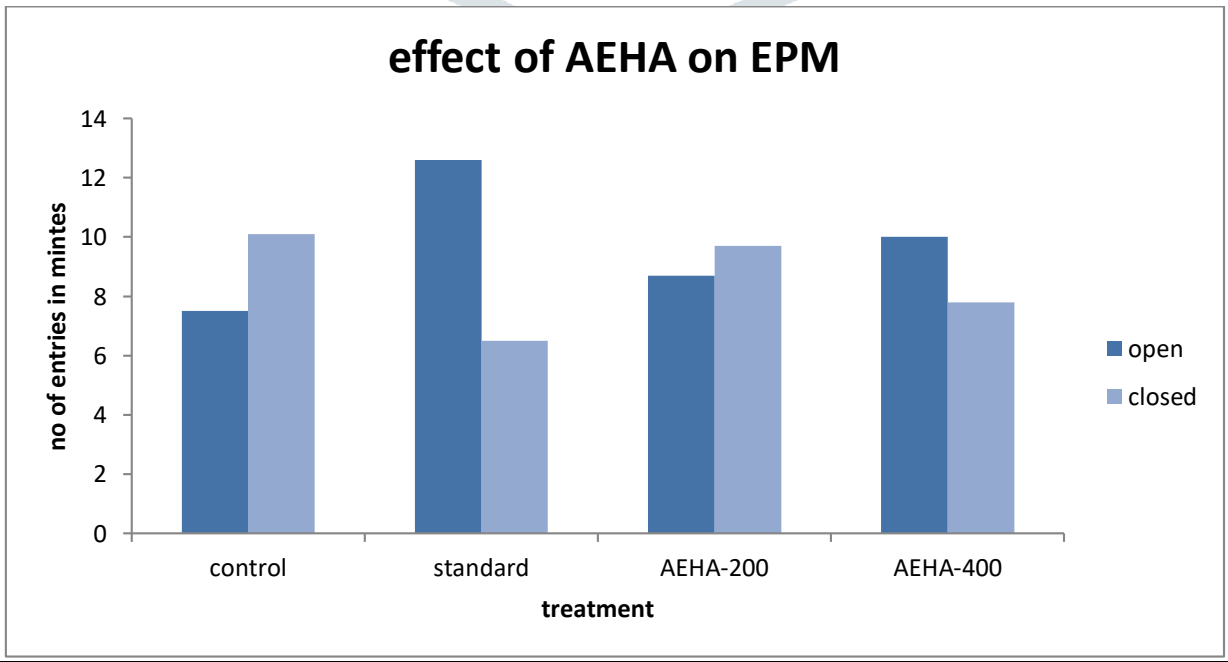


Fig 1: effect of AEHA on EPM

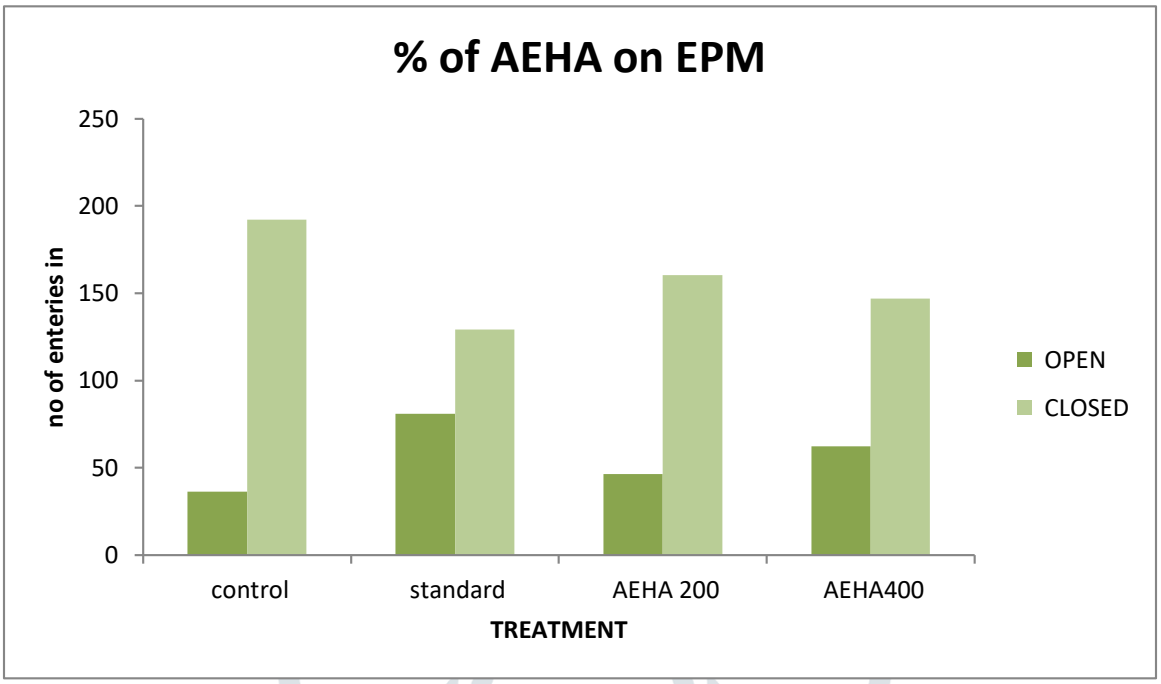


Fig 2: % of AEHA on EPM

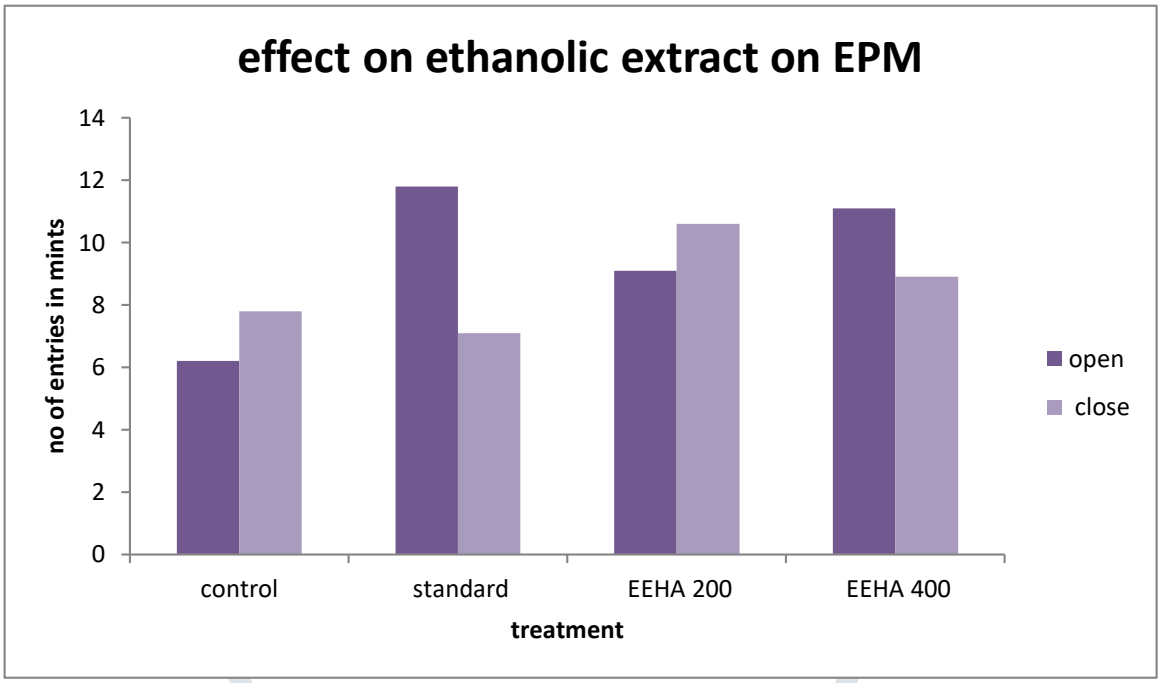
Table 2: effect of ethanolic extract on Elevated plus maze appartus

Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in min(mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	Saline(2%)	6.2± 0.4014	7.8± 0.9098	25.7± 0.9098	189.4± 3.416
II	Diazepam	5mg/kg	11.8± 0.5627***	7.1 ± 0.4216***	78.23±0.98042***	124.5± 2.301***
III	EEHA	200mg/kg	9.1± 0.3416	10.6± 0.4014	56.77± 1.256**	150 ± 2.414***
IV	EEHA	400mg/kg	11.1± 0.3416**	8.9± 0.3651***	59.33± 1.994***	135.4±1.713***

Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis was performed using Dunnettís test. *P < 0.05, **P < 0.01,

***P < 0.001 vs. control

Fig 3: **effect on ethanolic extract on EPM**



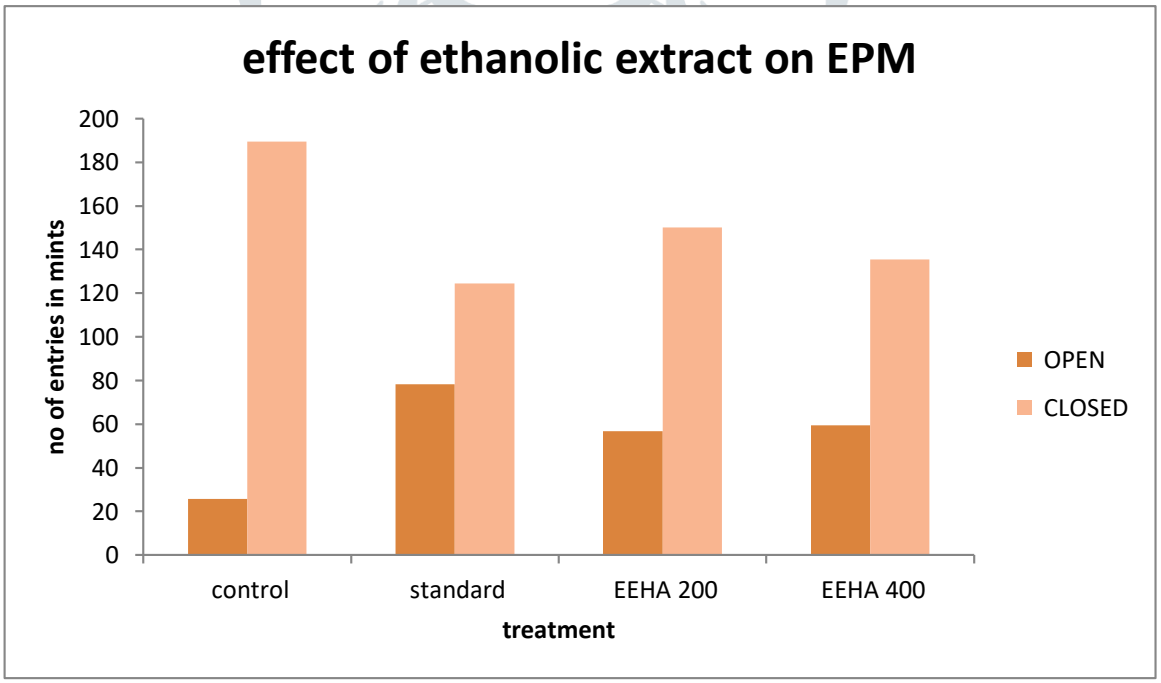


Fig 4: effect of ethanolic extract on EPM

DISCUSSION:

Anxious reaction is an adaptive reaction of an individual when confronted with danger or threat. Behavioral and physiological responses accompanying anxiety prepare an individual to react appropriately to such situation. One of the most widely used animal models for screening putative anxiolytic is the elevated plus-maze· The EPM is

considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform, moreover it is known that anxiolytic agent increases the frequency of entries and time spent in open arm of the EPM . In agreement with previously published reports, diazepam increased the percentage time spent on open arms and the number of entries on open arms. Total number of open arm entries and number of closed arm entries are usually employed as measures of general activity. In the present study it is noted that administration of AEMC prolonged the time spent in the open arms and the number of entries into open arms.

CONCLUSION

In pharmacological screening method, the *helianthus annus* seeds extraction when administered in mice shown less potent anxiolytic activity when compared to the standard drug, by using elevated plus maze and light/dark box.The phytochemical study it was proved that flavanoides, sesquiterpens, coumarin, terpinoids, are present. From the study it was shown that the Aqueous extract has shown more significant response when compare with control and standard it was proved that helianthus annus were shown to posses fewer side effects and anxiolytic properties in mice. the utilization of these plants in traditional medicine in Cameroon in the treatment of fever, agitations and anxiety.

BIBILOGRAPHY

- Chandra V, Singh A, Kapoor LD. Experimental cultivation of some essential oil bearing plants in saline soils, Matricaria chamomilla L. Perfum Essent Oil Rec. 1968;59:871.
- Chandra V. Cultivation of plants for perfumery industry at Lucknow. Indian Perfumer. 1973;16:40–4.
- Das M, Mallavarapu GR, Kumar S. Chamomile (Chamomilla recutita): Economic botany, biology, chemistry, domestication and cultivation. J Med Aromat Plant Sci. 1998;20:1074–109.
- Handa KL, Chopra IC, Abrol BK. Introduction of some of the important exotic aromatic plants in Jammu and Kashmir. Indian Perfumer. 1957;1:42–9.
- Issac O. 1st ed. Czecho-Slovakia: Prague press; 1989. Recent progress in chamomile research- medicines of plant origin in modern therapy.
- Ivens GM. Stinking mayweed. N Z J Agric. 1979;138:21–3.
- Kumar S, Das M, Singh A, Ram G, Mallavarapu GR, Ramesh S. J Med Aromat Plant Sci. 2001;23:617–23.
- Lawrence BM. Progress in Essential Oils. Perfume Flavorist. 1987;12:35–52.
- Svab J. New aspects of cultivating chamomile. Herba Polonica. 1979;25:35–9.
- Tyihak E, Sarkany-Kiss J, Verzar-Petri G. Phytochemical investigation of apigenin glycosides of Matricaria chamomilla. Pharmazie. 1962;17:301.
- Bradley P. The British herbal compendium. In: Bradley P, editor. 1st ed. London: British Herbal Medicine Association; 1992.

➢ Mann C, Staba EJ. The chemistry, pharmacology and commercial formulations of chamomile. In: Craker LE, Simon JE, editors. Herbs, spices and medicinal plants- recent advances in botany, horticulture and pharmacology. USA: Haworth Press Inc; 2002. pp. 235–80.