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Evaluate in Anxiolytic activity of Helianthus annus L. seeds of Ethanol and Aqueous extraction in Albino rats using light and dark methods

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Abstract

Anxiety disorders are the most prevalent mental health conditions. Although they are less visible than schizophrenia, depression, and bipolar disorder, they can be just as disabling. The diagnoses of anxiety disorders are being continuously revised. This approach is based partly on the need for a more comprehensive understanding of how biology, stress, and genetics interact to shape the symptoms of anxiety. Anxiety disorders can be effectively treated with psychopharmacological and cognitive– behavioral interventions. New developments are forthcoming in the field of alternative strategies for managing anxiety and for treatment-resistant cases. Additional treatment enhancements should include the development of algorithms that can be easily used in primary care and with greater focus on managing functional impairment in patients with anxiety.

Keywords: Anxiety, schizophreni , depression, and bipolar disorder.

I. INTRODUCTION

Anxiety disorders are present in up to 13.3% of individuals in the U.S. and constitutethe most prevalent subgroup of mental disorders.[1] The extent of their prevalence was first revealed inthe Epidemiological Catchments A ea study about 26 years ago.[2] Despite their widespread prevalence, these disorders have not received the same recognition as other major syndromes such as mood and psychotic disorders; in addition, the primary care physician is usually the principal assessor and treatment provider.[3],[4]As a result of this management environment, anxiety disorders can be said to account for decreased productivity, increased morbidity and mortality rates, and the growth of alcohol and drug abuse in a large segment of the population.[5,7]

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.(8)

Sunflower is the common name 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.(9)

Fig 1: ***Helianthus annuus***

Although Native Americans domesticated the plant and selected for plants with single heads and larger seeds, its initial use after being introduced into Europe was primarily as an ornamental plant in gardens.(10)

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.(11)

Helianthus annuus is rich in medicinal properties. The understanding of these properties will help us to better utilize this herb.
Below is given medicinal properties along with the meaning.

Anti-inflammatory: Reducing inflammation by acting on body mechanisms.

Antipyretic/antifebrile/febrifuge: Effective against fever.

Astringents: Constrict tissues; styptic.

Collection of Plant Material:

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

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.

Drying: The collected leaves were dried for 14 days at room temperature (28-37 ^{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 70 ^{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 InVitro as says.

Preliminary Phytochemical Screening:
The collected extracts were subjected for phytochemical screening using freshly prepared reagentsto analyze the present phytoconstituents in extracts. The extracts were analyzed for the detection of alkaloids, glycosides, flavonoids, proteins, saponins, phenols, terpenoids, amino acids,

steroids, carbohydrates and tannins. **(*Kokate et al; 2017*)**

Approval of protocol:
All the experimental procedure and protocols used in the present study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of control and supervision of experiments on animals (CPCSEA). For this, study protocol (form no-Form B of CPCSEA) was prepared and submitted to the institutional ethics committee for approval to carryout experiments on animals.

The animals were housed in polyacrylic cages (38X23X10 cm) with not more than four animals percage. 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 ± 2ºC. The animals were allowed to free access to standard diet and water ad libitum and allowed to acclimatize for one week before the experiments.

Acute toxicity studies of leave extracts were studied in female mice according to the guidelines for organization of economic cooperation and development(OECD 421). 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).***

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).

For aqueous

a) <u>Light and dark method</u>
Albino rats were divided into four groups of 5 animals each.

GroupI–Control(2%saline)

Group II – Standard drug(diazepam- 5mg/kg i.p)GroupIII–AEHA (200mg/kg)
GroupIV– AEHA(400mg/kg)

Group 1- Control (2%Saline)

Group II – Standard drug(diazepam- 5mg/kg i.p)Group III– EEHA (200mg/kg)
Group IV– EEHA(400mg/kg)

Light Dark Method:

Fig 2: light and dark model Description:

- **Light dark test**: The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent.

- The mice were allowed to move from one box to the other through an open door betweenthe two boxes. A 100W bulb placed 30 cm above the floor of the transparent box was the only light source in the room. A mouse was put into the light box facing the hole.
- The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

II. REQUIREMENTS:

Animals: Albino rats (150-230gms)
Drugs: standard drug(Diazepam(5mg/kg.,i.p)
Equipment: elevated plus maze apparatus and light and dark method.

Apparatus:
1. Round bottomed flask. 2. Condenser.3. Heating metel.4. Beakers.5. Stands 6. Water 7. Filter paper.
8. Stop watches. 9. Thermometer.10. Syringes. 11. Weighing balance.12. Absorbent cotton and oralfeeding tube.
5.Plant Materials:helianthus annuus seeds(coarse powder).

Procedure: Helianthus annus seed: Powder of Helianthus seeds

Figure 3: Powder of Helianthus seeds

- The light and dark transition test is based on the innate aversion of mice to brightly illuminated areas and on the spontaneous exploratory behavior of mice in response to mild stressors, that is, novel environment and light. The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug induced increase in behavior in the white part of a two compartment box, in which a large white compartment is darkened, is suggested as an index of anxiolytic activity
- The apparatus for light/dark transition test consist of two compartments: one light area (20x 10 x14) was painted black. The two compartments were separated by a partition with a tunnel to allow passage from one compartment to the other. The experiments were performed between 9:00 and14:00. Animal was placed in the center of the light area with its black opening. The following parameter were recorded during 5 min. Latency time for the first crossing to the dark compartment, the number of transition between the light and the dark compartment(tunnel crossing), the total time spent in the light compartment. The apparatus was cleaned thoroughly between trials.

Results are expressed as mean ± standard error of the mean (S.E.M.). All data are subjected to analysisof variance (ANOVA) followed by Dunnetísìtî test. P values <0.05(90% confidence limit)was considered statistically significant.

III. RESULTS:

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

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

Percentage yield = Weight Obtained Of extracts X 100

Weight of crude 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%

IV. RESULT OF PHYTOCHEMICAL SCREENING:

Table 2: Result of Preliminary phytochemical screening of variousextract of *helianthusannus* seeds

Phytochemical screening	Aqueous extract of *Helianthus Annus l*	Ethanol extract of *Helianthus Annus I*
Carbohydrates	+	+
Glycosides	+	-
Flavonoids	-	+
Saponins	+	–
phenols	-	–
Alkaloids	+	+
Proteins and aminoacids	-	–
Phenol and phenolic compounds	-	–
Terpenoids	-	–
Tannins	-	+

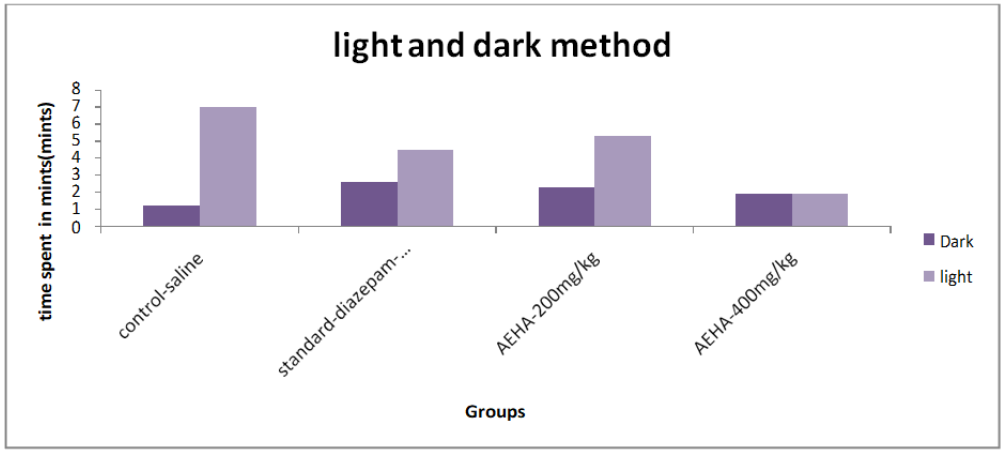
Toxicity study:

In the current exploration, the Aqueous and ethanol extracts of *helianthus seeds l* were levied for studies of acute toxicity. For the determination of LD50dose, ethanol and aqueous extract of *helianthus seeds l* In the current exploration, the Aqueous and ethanol extracts of *helianthus seeds l* were levied for studies of acute toxicity. For the determination of LD50dose, ethanol and aqueous extract of *helianthus seeds l* was given up to dose o f2 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 the current investigation.

Table No 3: Effect of AEHA on Light and Dark transition model:

			Dose spent in min(mean±SEM)		Number of entries(mean±SEM)	
Group no	**Drug treatment**	**Dose (mg/kg)**	**Dark**	**Light**	**Dark**	**Light**
I	Control	Saline	1.2± 0.3333	7±0.2236	4.50±0.2236	1.167±0.1167
II	Diazepam	5mg/kg	2.6±0.3073***	4.5±0.3651*	12.50±0.5627***	5.333±0.3333***
III	AEHA	200mg/kg	2.3±0.3333	5.3±0.2500	7.167±0.3073***	1.33 ±0.3333
IV	AEHA	400mg/kg	1.9± 0.3073***	1.9±0.3416	8.33±0.3333***	2.833±0.3073**

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 Dunnett ís test. * P<0.05, ** P<0.01,
*** P<0.001 vs.control



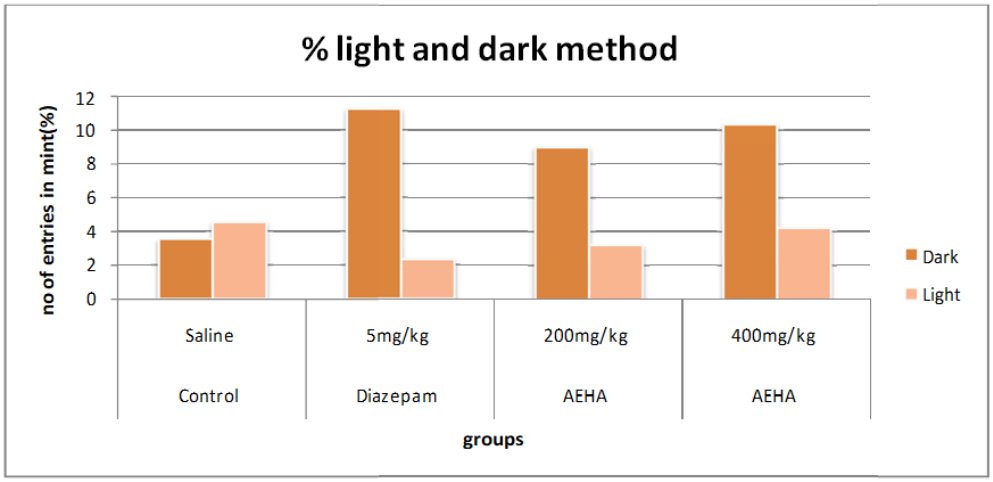


Fig 5: percentage of light and dark model in animal

Table4: Effect of EEHA on Light and Dark transition model:

			Dose spent in min(mean±SEM)		Number of entries(mean±SEM)	
Group no	**Drug treatment**	**Dose (mg/kg)**	**Dark**	**Light**	**Dark**	**Light**
I	Control	Saline	1.5± 0.3333	6±0.2236	3.55±0.2236	4.55±0.1167
II	Diazepam	5mg/kg	3.2±0.3073***	3.5±0.3651*	11.25±0.5627***	2.33±0.3333***
III	EEHA	200mg/kg	2.5±0.3333	5.5±0.2500	8.99±0.3073***	3.22 ±0.3333
IV	EEHA	400mg/kg	2.1± 0.3073***	7.6±0.3416	10.33±0.3333***	4.234±0.3073**

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 Dunnettístest. *P<0.05, **P<0.01,
***P<0.001 vs.control.

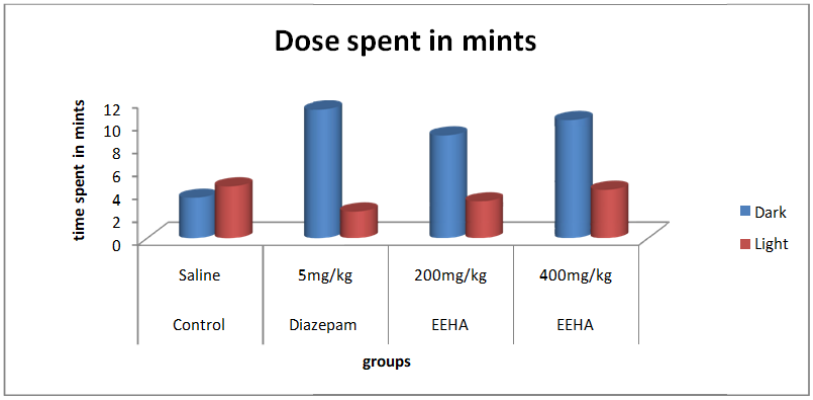


Fig 6: Ethanol Extract in Light and Dark Models

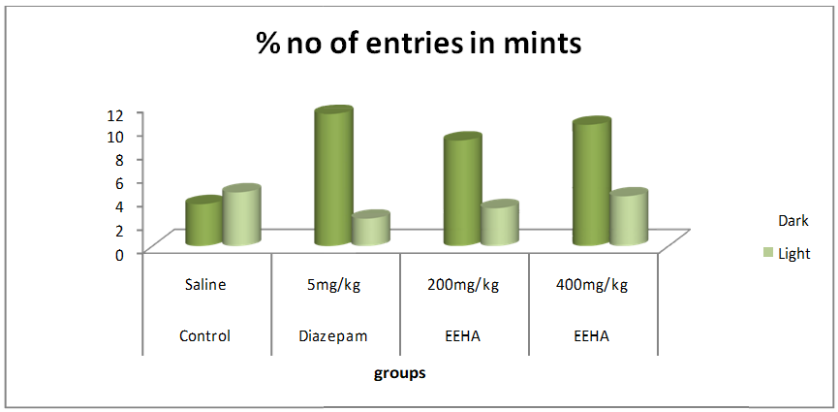


Fig 7:% of light and dark model in rats

In Light and dark method, animals treated with three doses of AEMC (200 and 400 mg/kg) &diazepam showed reduced time spent but increase in number of entries in dark chamber and With concomitant increase in time & number of entries in light chamber when compared with controls. Animals treated with high dose an dm o d e r a t e(200 a n d 400 mg/kg) shows more significant results.

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.
The light/dark box is also widely used for rodents as a model for screening anxiolytic oranxiogenic drugs, based on theinnate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light[17]. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolyticaction[18]. The present study showed that AEHA could increase the time in the light area, suggesting again that AEHA possesses anxiolytic properties.

V. CONCLUSION:

Inpharmacological 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 inmice. the utilization of these plants in traditional medicine in Cameroon in the treatment of fever, agitations and anxiety.

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