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A STUDY ON ANXIOLYTIC ACTIVITY OF AQUEOUS EXTRACTS OF *Matricaria chamomilla L* IN ELEVATED PLUZ MAZ APPARATUS AND LIGHT AND DARK CHAMBER IN ALBINO MICE

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ABSTRACT:

This study was performed to investigate the anxiolytic effects of alcoholic extract of *Matricaria chamomilla L* (AEMC) in mice using the elevated plus-maze model (EPM), light dark model and hole board test. The extract administered orally in three different doses of 250mg/kg, 500mg/kg and 750mg/kg, were able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze, also increases the time spent by mice in the illuminated side of the light and dark test, dose of 500mg/kg and 750mg/kg showed more significant increase in nose poking and decrease locomotion in hole board test, in comparison with control animals. This effect was comparable to that of the diazepam (1.0mg/kg p.o.). These results indicate that AEMC is an effective anxiolytic agent.

Keyword: Anxiolytic-like effect, *Matricaria chamomilla L*, Elevated plus maze, Light and dark chamber, Diazepam.

Introduction:

Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints, and rumination.[1] It is the subjectively unpleasant feelings of dread over anticipated events, such as the feeling of imminent death.[2] Anxiety is not the same as fear, which is a response to a real or perceived immediate threat,[3] whereas anxiety is the expectation of future threat.[3] Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing.[4] It is often accompanied by muscular tension,[3] restlessness, fatigue and problems in concentration. Anxiety can be appropriate, but when experienced regularly the individual may suffer from an anxiety disorder.[3]

People facing anxiety may withdraw from situations which have provoked anxiety in the past.[5] There are various types of anxiety. Existential anxiety can occur when a person faces angst, an existential crisis, or nihilistic feelings. People can also face mathematical anxiety, somatic anxiety, stage fright, or test anxiety. Social anxiety and stranger anxiety are caused when people are apprehensive around strangers or other people in general. Furthermore, anxiety has been linked with physical symptoms such as IBS and can heighten other mental health illnesses such as OCD and panic disorder. The first step in the management of a person with anxiety symptoms is to evaluate the possible presence of an underlying medical cause, whose recognition is essential in order to decide its correct treatment.[6][7] Anxiety symptoms may be masking an organic disease, or appear associated or as a result of a medical disorder.[6][7][8][9]

Collection of Plant Material:

The fresh flowers of *Matricaria chamomilla* L were collected in the month of Nov – jan from the coastal region of Andhra Pradesh.

Identification and Authentication:

The collected plant part (flowers) of *Matricaria chamomilla* 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 *Matricaria chamomilla* L For further study the extracts were dissolved in double distilled water for further In Vitro assays.

a) Elevated pluz maz apparatus

Albino mice's were divided into five groups of 6 animals each.
Group I – Control (2% saline)
Group II – Standard drug(diazepam- 5mg/kg i.p)
Group III – AEMC (200mg/kg p.o)
Group IV – AEMC (400mg/kg p.o)
Group V-AEMC

b) Light and dark method

Albino mice's were divided into five groups of 6 animals each.
Group I – Control (2% saline)
Group II – Standard drug(diazepam- 5mg/kg i.p)
Group III – AEMC (250mg/kg)
Group IV – AEMC (500mg/kg)
Group V-AEMC(750 mg/kg)

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:

Extraction:

Size reduced powder of flowers of *matricaria chamomile 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: Percentage yield of the collected extracts

s.no	Extract	Weight taken (Grams)	Percentage yield
1	Aqueous extract of ***matricaria chamomile l***	200	34%

Result of Phytochemical Screening:

Table 16: Result of Preliminary phytochemical screening of various extract of *matricaria chamomile l* flowers

Phytochemical	Aqueous extract of *matricaria chamomile l*
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 *matricaria chamomile l* were levied for studies of acute toxicity. For the determination of LD_{50} dose, Methanol extract of *matricaria chamomile l* was given up to dose of 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:

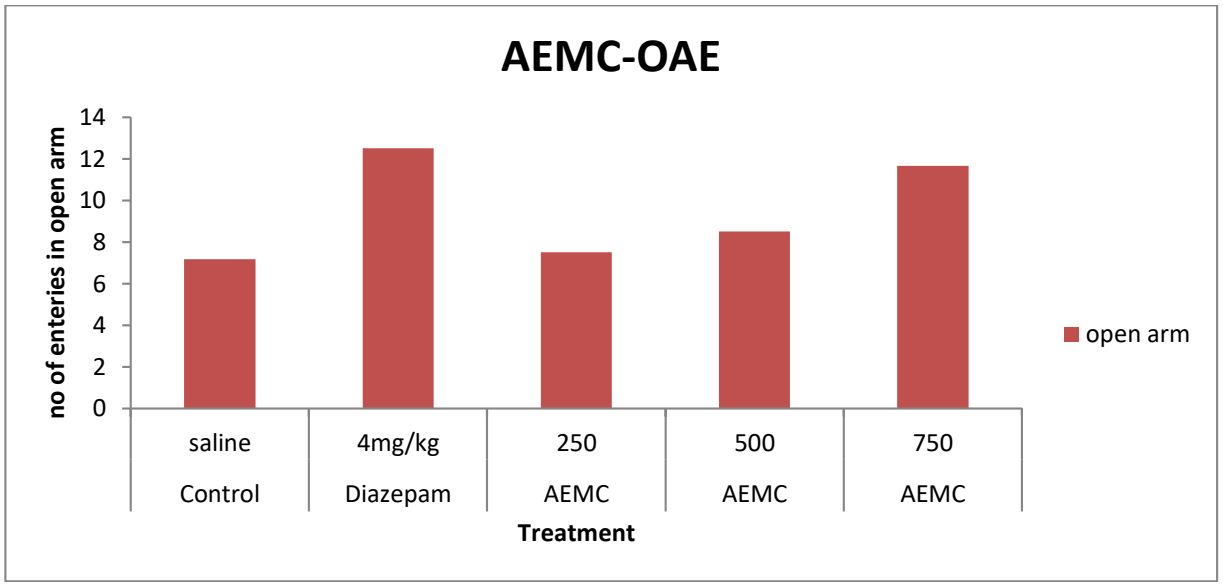
Effect of AEMC on Elevated plus maze:

In EPM saline treated animals the time spent & entries in the open and closed arms, were compared with AEMC extract at the dose of 250mg/kg, 500mg/kg and 750mg/kg & also Diazepam (1mg/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 (Graph 1& 3). Furthermore, AEMC 250, 500 and 750 mg/kg had decrease in time spent and number of entries in closed arm (graph 2 &4) as Diazepam showed a significant ($p<0.05$) in elevated plus-maze.

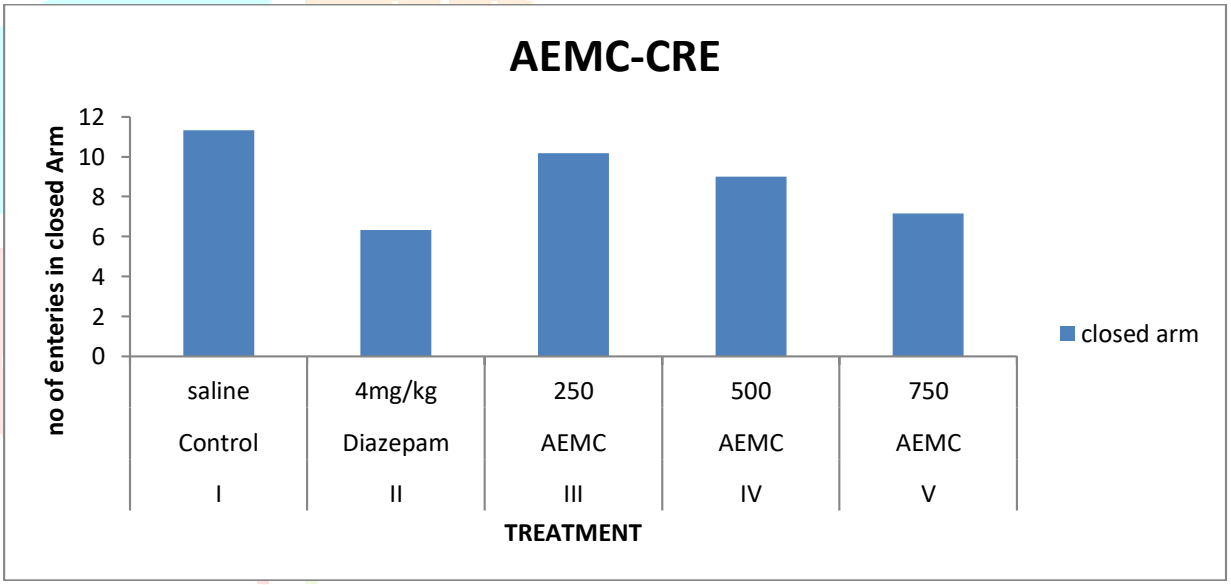
Table No.1: Effect of AEMC on EPM paradigm in mice

Group No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in sec (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	saline	7.167 ± 0.4014	11.33 ± 0.9098	36.17± 0.9098	192.0 ± 3.416
II	Diazepam	4mg/kg	12.50± 0.5627***	6.33 ± 0.4216***	80.83±0.98042***	129.2± 2.301***
III	AEMC	250	7.50 ± 0.3416	10.17 ± 0.4014	46.33± 1.256**	160.2 ± 2.414***
IV	AEMC	500	8.50 ± 0.3416**	9.0 ± 0.3651***	62.33± 1.994***	147.00±1.713***
V	AEMC	750	11.67 ± 0.7601***	7.167 ± 0.3073***	79.17± 1.493***	136.2 ± 2.482***

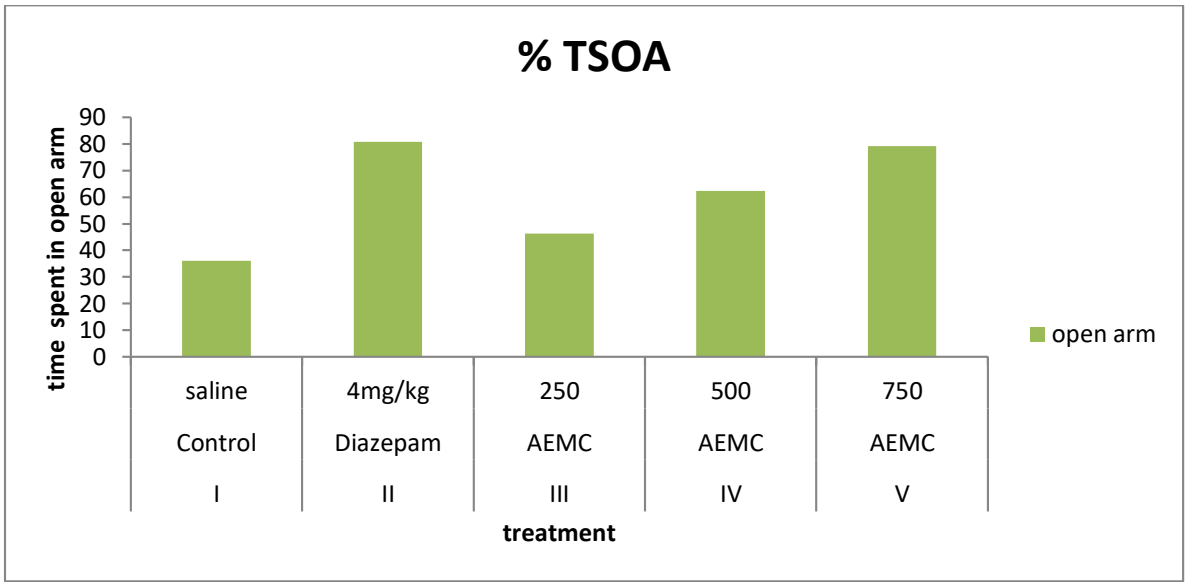
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis wasperformed using Dunnettís test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control



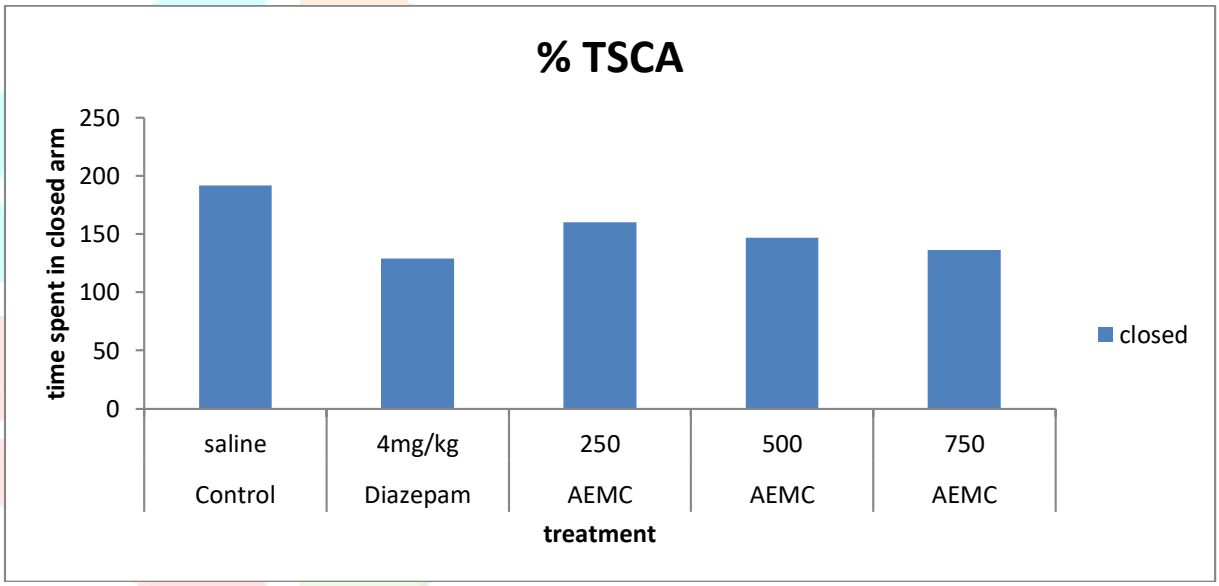
Graph 1: No. of entries in open arm in EPM



Graph 2: No. of entries in closed arm in EPM



Graph 3: time spent in open arm in EPM

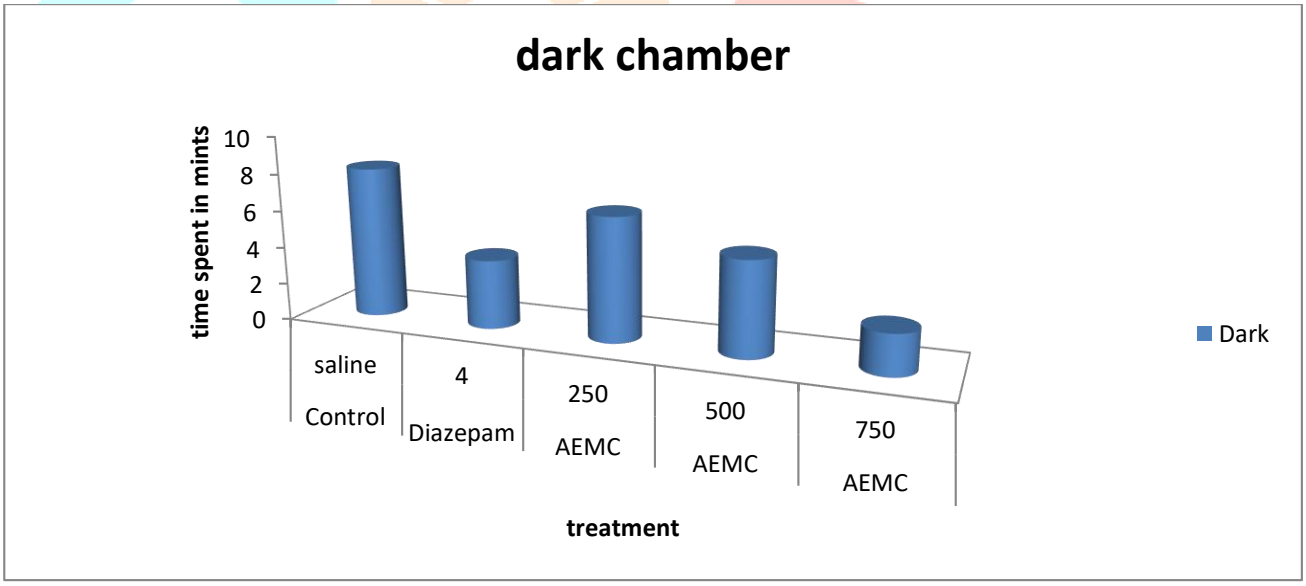


Graph 4: time spent in closed arm in EPM

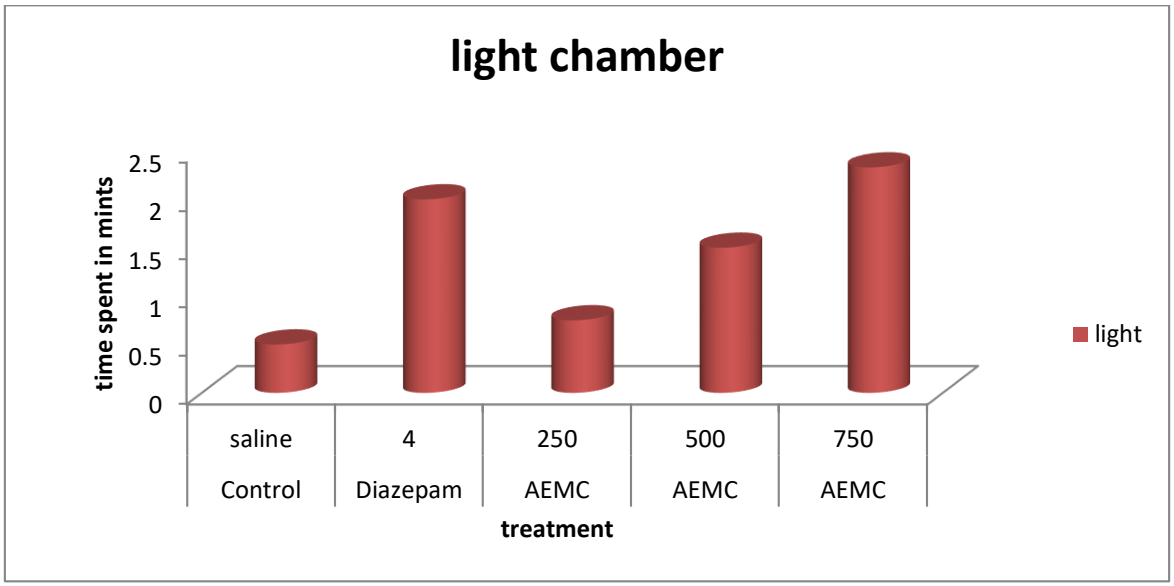
Table No 2: Effect of AEMC on Light and Dark transition model :

Group No.	Drug Treatment	Dose (mg/kg)	Time spent in min(Mean±SEM)		Number of Entries(Mean±SEM)	
			Dark	Light	Dark	Light
I	Control	saline	7.33 ± 0.3333	0.5 ± 0.2236	4.50 ± 0.2236	1.167± 0.1167
II	Diazepam	4	3.833± 0.3073***	2.0 ± 0.3651*	12.50 ± 0.5627***	5.333 ± 0.3333***
III	AEMC	250	6.667± 0.3333	0.75 ± 0.2500	7.167± 0.3073***	1.33 ± 0.3333
IV	AEMC	500	5.167 ± 0.3073***	1.5 ± 0.3416	8.33 ± 0.3333***	2.833 ± 0.3073**
V	AEMC	750	3.167± 0.4773***	2.33 ± 0.6146**	11.83 ± 0.3073***	4.167 ± 0.3073***

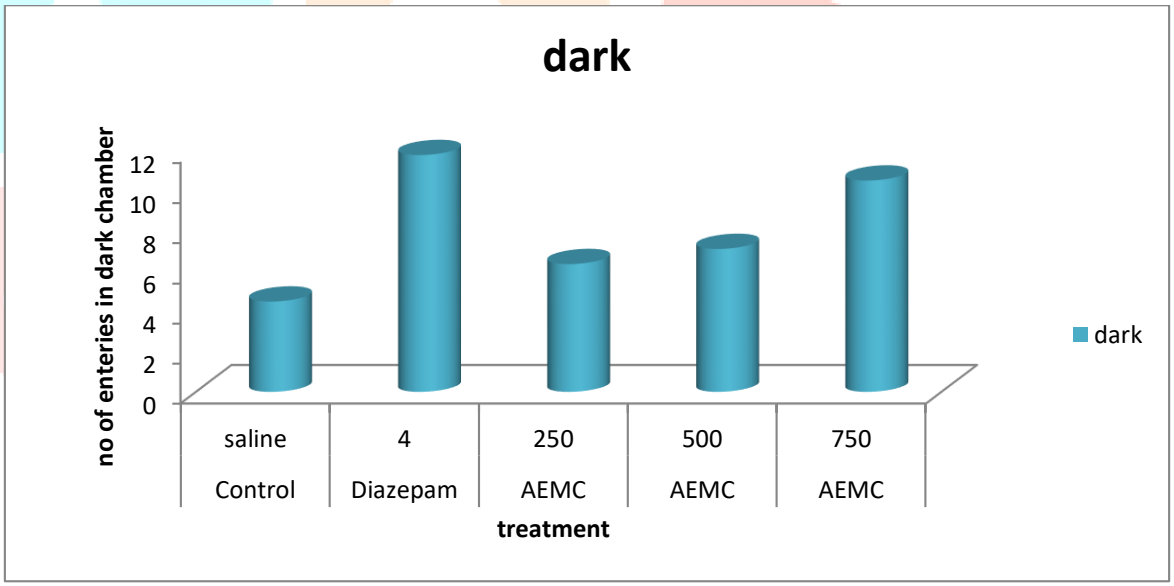
Values were mean ± S.E.M. for (n=6) expressed as the time (in sec) of 6 animals in each group. Data analysis wasperformed using Dunnettís test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control



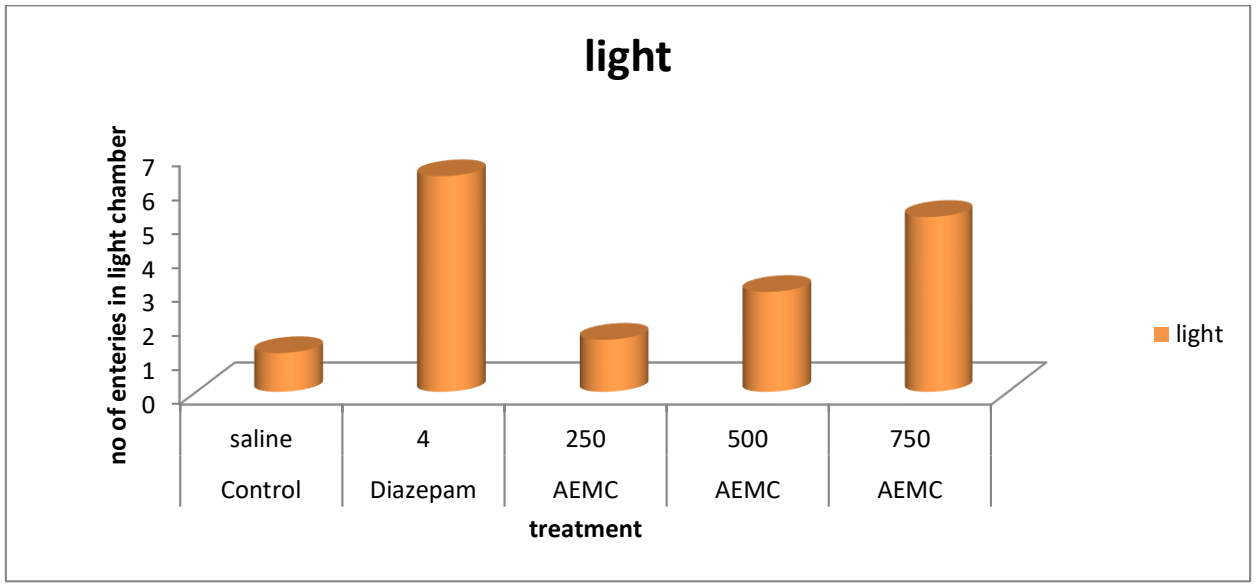
Graph 5: Time spent in dark chamber in LDT



Graph 6: Time spent in light chamber in LDT



Graph 7: No. of entries in dark chamber in LDT



Graph 8: No. of entries in light chamber in LDT

Effect of AEMC on Light dark model

In LDT, (Table No. 2) animals treated with three doses of AEMC (2 5 0 , 500 and 750 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 and moderate (5 0 0 and 750 mg/kg) shows more significant results when compared with low dose (250 mg/kg).

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

CONCLUSION

In pharmacological screening method, the matricaria chamomile flower 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 matricaria chamomile 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.

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