



# INVESTIGATION OF EFFECTIVENESS ON ETHANOLIC AND AQUEOUS EXTRACT OF CINNAMOMUM CASSIA BARK IN ANXIOLYTIC ACTIVITY IN EXPERIMENTAL ALBINO RATS USING LIGHT AND DARK METHODS

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## ABSTRACT:

The present study is aimed to evaluate the anxiolytic activity of ethanol and aqueous extract of cinnamomum cassia bark using elevated plus maze test (epm) model in rats. ethanol and aqueous extract of cinnamomum cassia bark 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:** CINNAMOMUM CASSIA BARK ,Anxiolytic, Elevated plus maze test

## INTRODUCTION:

### ANXIETY

Anxiety is a naturally occurring emotion and an innate response of the body to environmental stimuli. Mild to moderate anxiety would increase the 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)

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#### **Anxiolytics:**

Anxiolytics are a class of medications used to prevent or treat anxiety symptoms or disorders. They're sometimes called anti-anxiety medications or minor tranquilizers. Anxiolytic medications are habit-forming and can lead to dependency or a substance use disorder. For this reason, they're often only prescribed for a short amount of time. There are different types of anxiety disorders. For some types, your doctor might use antidepressant medication first. If those don't work, they might try anxiolytics. There are different types of anxiolytic medications that work in different ways. Benzodiazepines.(2) These medications are called central nervous system depressants. It's not entirely clear how benzodiazepines work, but they raise levels of an amino acid in your brain called gamma-aminobutyric acid (GABA). GABA blocks other activity in your brain, which helps you feel calm and can make you sleepy.



**Fig 1: cinnamomum cassia**

cinnamomum cassia, called Chinese cassia or Chinese cinnamon, is an evergreen tree originating in southern China and widely cultivated there and elsewhere in South and Southeast Asia.[3] It is one of several species of Cinnamomum used primarily for its aromatic bark, which is used as a spice. The buds are also used as a spice, especially in India, and were used by the ancient Romans(4)

Geographical source: Sri Lanka, Malabar Coast of India, Jamaica and Brazil ect.Cultivation and Collection:- It is generally cultivated by seed propagation method but sometimes plant cuttings are also preferred. It mainly needs sandy or siliceous soil which should be rich in humus. The other requirements for its better cultivation are altitude (800 to 1000 meter) and annual rainfall (200 to 250 cm). It is shade loving plant.(5)

### **Chemical Constituents:-**

Cinnamon bark contains polycyclic diterpenes and proanthocyanidinoid oligomers. It contains volatile oils (0.5 to 1 percent), phlobatannins (1.2 percent), mucilage, calcium oxalate, starch and mannitol (responsible for sweetish taste).(6) The cinnamon oil obtained from distillation method which is light yellow in colour and upon storage changes to reddish in colour. The essential oil (5 to 20 ml/kg) is composed of phenylpropane derivatives. Cinnamon oil mainly contains cinnamaldehyde (60 to 70 percent), eugenol (5 to 10 percent), benzaldehyde, cuminaldehyde and other terpenes such as phellandrene, pinene, cymene, caryophyllene.(7).

### **Chemical Test:-**

To a drop of volatile oil add a drop of ferric chloride solution, a pale green colour develops (cinnamaldehyde produces brown colour and eugenol gives blue colour which results in the formation of pale green colour).(8)

### **Uses:-**

The drug is used as aromatic stimulant, antibacterial, antifungal, antiseptic, carminative, stomachic and astringent. Commercially, it is also used as spice, condiment, in candy preparation, dentrifices and perfumery. Cinnamon oil is used in urinary infection and food technology. Cinnamon oil and cinnamaldehyde are irritating to skin and mucous membranes. They cause allergic reactions like urticaria or edema of the face and lips.(9).

## **MATERIALS AND METHODS:**

### **Collection of Plant Material:**

The fresh bark of *Cinnamomum Cassia L* were collected in the month of Nov – jan from the coastal region of Andhra Pradesh.

### **Identification and Authentication:**

The collected plant part (bark) of *Cinnamomum Cassia 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 bark were dried for 7 days at room temperature (27-37 °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 °C for 24 hours, using soxhlet apparatus. The extracts were then filtered and dried under vacuum.

## QUALITATIVE ANALYSIS:

### Preliminary Phytochemical Screening:

The collected extracts were subjected for phytochemical screening using freshly prepared reagents to analyze the present phytoconstituents in extracts. The extracts were analyzed for the detection of alkaloids, glycosides, flavonoids, proteins, amino acids, carbohydrates and tannins. (10)

### 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  $60 \pm 5\%$  and an ambient temperature of  $25 \pm 2^\circ\text{C}$ . 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 (11).

**Table 1: AQUEOUS EXTRACTS FOR LIGHT AND DARK METHOD**

S.no	GROUPS	Dose (mg/kg)
1	GROUP-1-CONTROL	SALINE 2%
2	GROUP-II-STANDARD	DIAZEPAM-5mg/kg)
3	GROUP-III- TEST	AECC-200mg/kg
4	GROUP-IV- TEST	AECC-400mg/kg

**Table 2: ETHANOL EXTRACTS FOR LIGHT AND DARK METHOD**

S.no	GROUPS	Dose (mg/kg)
1	GROUP-1-CONTROL	SALINE 2%
2	GROUP-II-STANDARD	DIAZEPAM-5mg/kg)
3	GROUP-III- TEST	EECC-200mg/kg
4	GROUP-IV- TEST	EECC-400mg/kg

**STATISTICALANALYSIS****Figure:3 Light and dark method**

## 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 between the 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.

### REQUIREMENTS:

**1. Animals:** Albino rats (120-150gms)

**2. Drugs:** standard drug (Diazepam (5mg/kg, i.p))

**3. Equipment:** elevated plus maze apparatus and light and dark method.

**4. Apparatus:** 1. Round bottomed flask. 2. Condenser. 3. Heating metal. 4. Beakers. 5. Stands. 6. Water. 7. Filter paper. 8. Stop watches. 9. Thermometer. 10. Syringes. 11. Weighing balance. 12. Absorbent cotton and oral feeding tube

Results are expressed as mean  $\pm$  standard error of the mean (S.E.M.). All data are subjected to analysis of variance (ANOVA) followed by Dunnett's *t* test. P values  $<0.05$  (95% confidence limit) was considered statistically significant.

**Results and Discussion:****Extraction:**

Size reduced powder of bark of *Cinnamomum Cassia* were extracted separately by Soxhlet extraction technique with alcohol and ethanol (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.3**

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

**Table 3: Percentage yield of the collected extracts**

S. no	Extract	Weight taken (Grams)	Percentage yield
1	AQUEOUS EXTRACT OF <i>Cinnamomum Cassia</i>	500	42%
2	Ethanol extract of <i>Cinnamomum Cassia</i>	500	35%

**Table 4: Result of Preliminary phytochemical screening of various extract of *Cinnamomum Cassia* bark**

Phytochemical	Aqueous extract of <i>Cinnamomum Cassia</i>	Ethanol extract of <i>Cinnamomum Cassia</i>
Carbohydrates	+	+
Glycosides	+	+
Flavonoids	+	+
Saponins	-	+
Alkaloids	+	+

<b>Proteins and amino acids</b>	-	-
<b>Phenol and phenolic compounds</b>	+	+
<b>Phytosterol</b>	+	+

**Note:** + sign indicate the presence; - sign indicate the absence

### Toxicity study:

In the current exploration, the Aqueous extracts of *Cinnamomum cassia* (bark) were levied for studies of acute toxicity. For the determination of LD<sub>50</sub> dose, Methanol extract of *Cinnamomum cassia* was given up to dose of 2 gm/kg b.w. and extracts did not exhibit any sort of mortality, that's why 1/5<sup>th</sup> (400mg), 1/10<sup>th</sup> (200mg) of most dose given were preferred for the current investigation.

**Table No.10: Effect of AECC on LIHGT AND DARK MODELS in albino rats**

s.no	Treatment	mg/kg	Number of Entries in time spent		Time spent in min (mean±SEM)	
			light	Dark	Light	Dark
<b>I</b>	<b>Control</b>	<b>Saline(2%)</b>	8± 0.3333	1.5±0.2236	4.50±0.2236	1.167±0.1167
<b>II</b>	<b>Diazepam</b>	<b>5mg/kg</b>	3.7±0.3073***	2.6±0.3651*	12.50±0.5627***	5.333±0.3333***
<b>III</b>	<b>AECC</b>	<b>200mg/kg</b>	5.3±0.3333	2.3±0.2500	7.167±0.3073***	1.33±0.3333
<b>IV</b>	<b>AECC</b>	<b>400mg/kg</b>	6.7± 0.3073***	1.9±0.3416	8.33±0.3333***	2.833±0.3073**

Values were mean±S.E.M. for (n=5) expressed as the time (in sec) of 5 animals in each group. Data analysis was performed using Dunnett's test. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001 vs. control

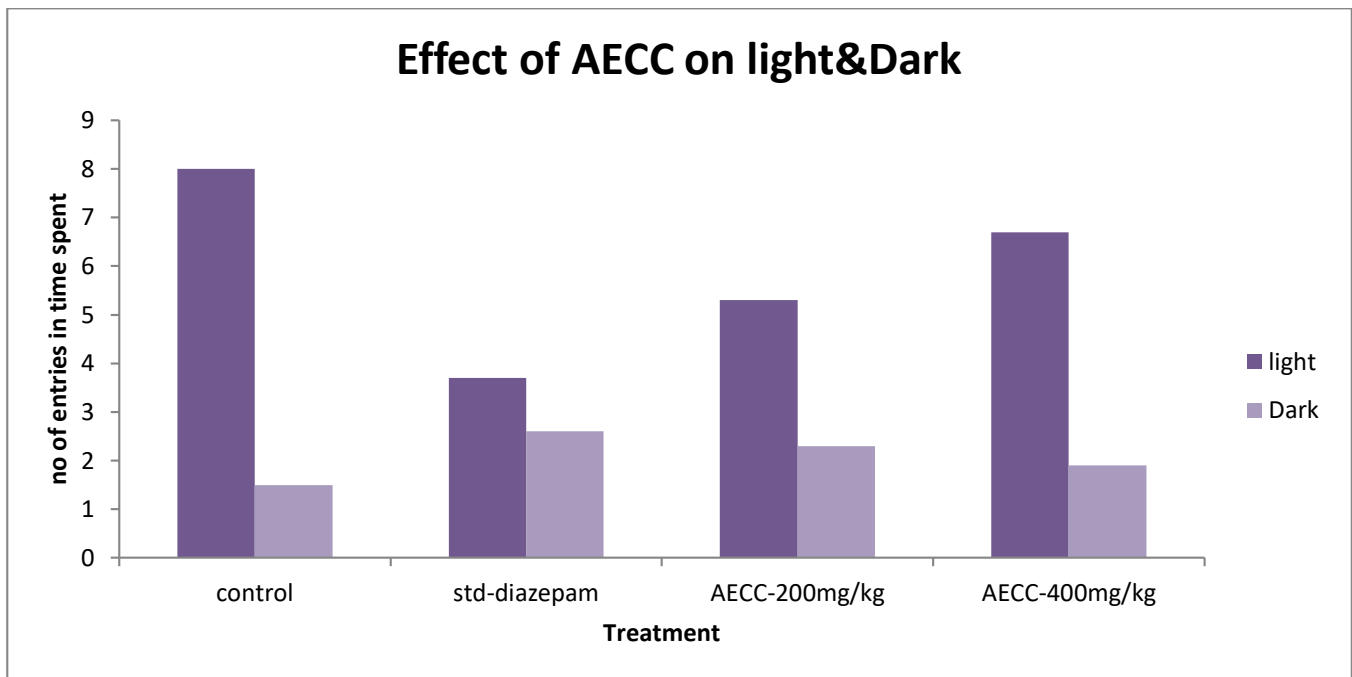


Fig 11: Effect of AECC on light and dark

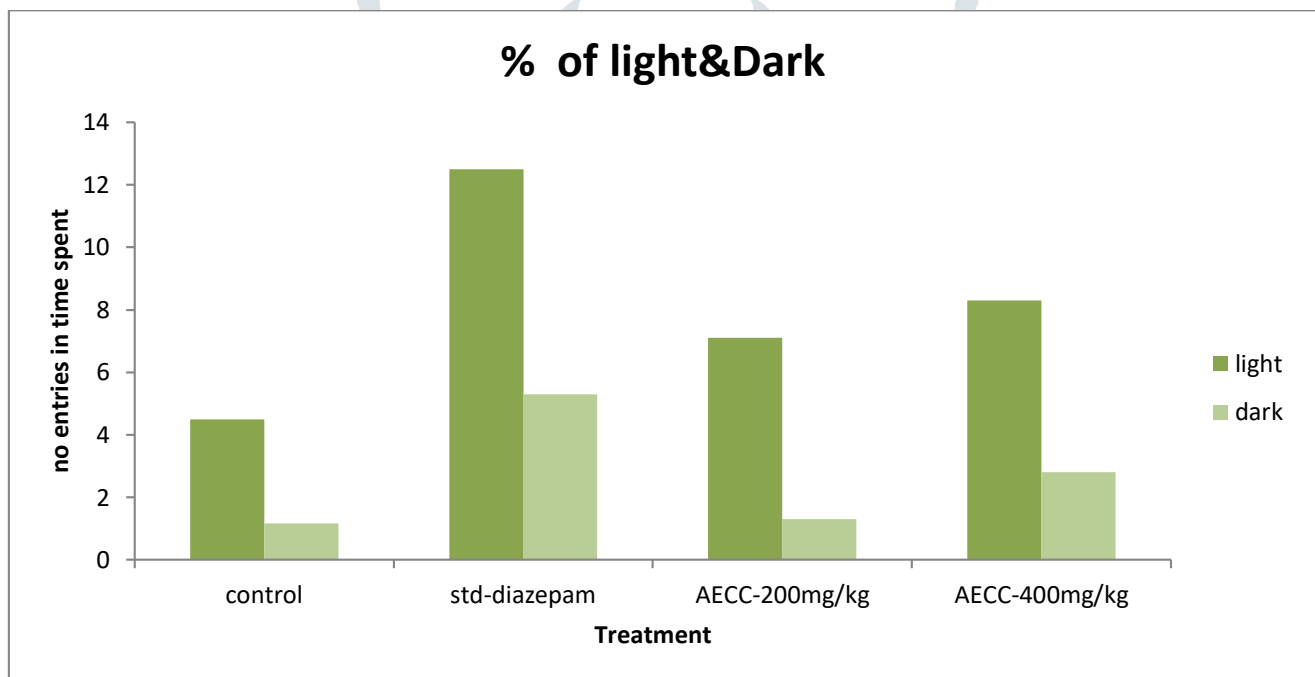


Fig 12: percentage of light and dark

TableNo.11:Effect of EECC on LIHGT AND DARK MODELS in abino rats

s.no	Treatment	mg/kg	Number of Entries in time spent		Time spent in min (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	Saline(2%)	7± 0.3333	1.5±0.2236	4.50±0.2236	1.167±0.1167
II	Diazepam	5mg/kg	4.9±0.3073***	1.9±0.3651*	12.50±0.5627***	5.333±0.3333 ***
III	EECC	200mg/kg	4.3±0.3333	1.8±0.2500	7.167±0.3073***	1.33 ±0.3333
IV	EECC	400mg/kg	7.6± 0.3073***	2.7±0.3416	8.33±0.3333***	2.833±0.3073 **

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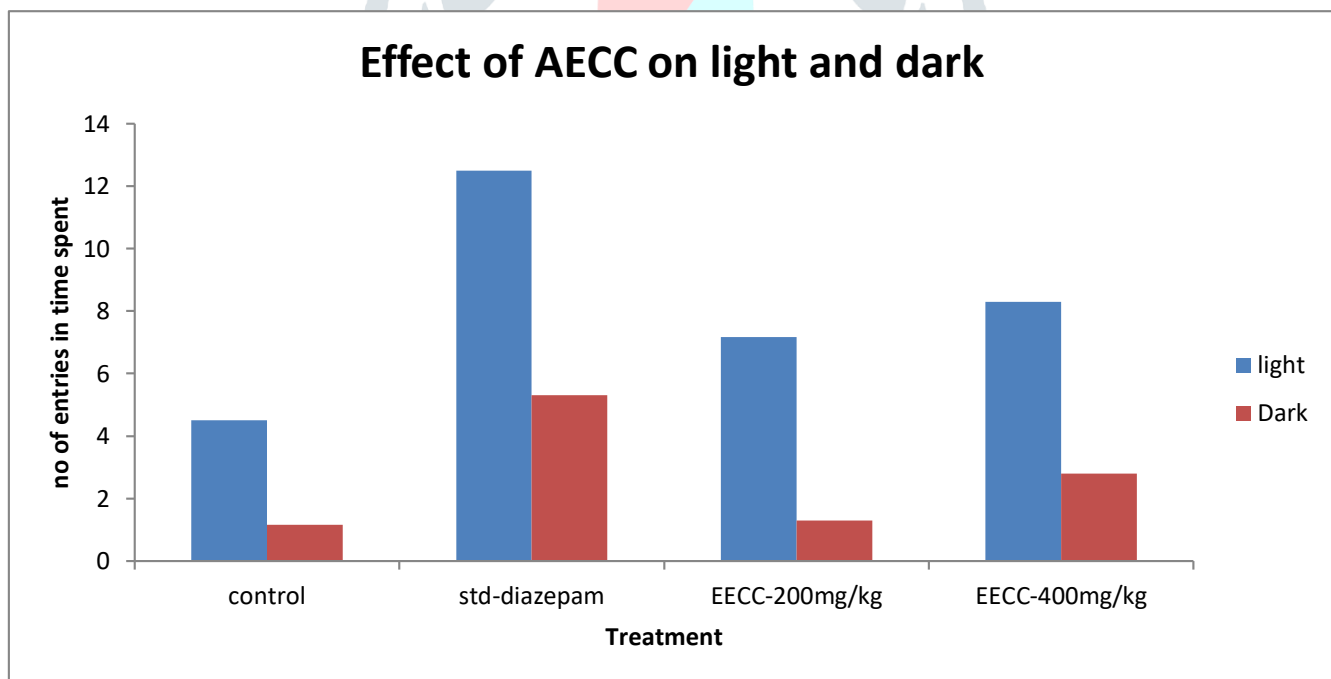
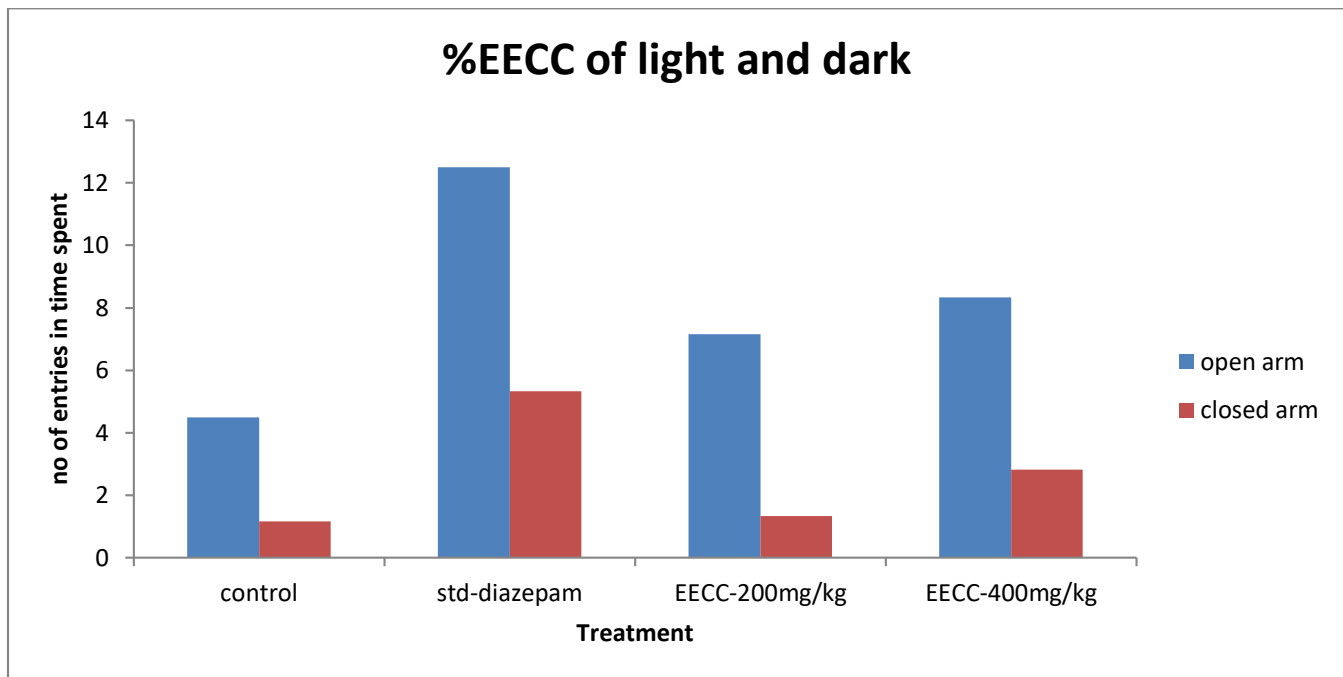


Fig 13: Effect of AECC on light and dark



**FIG 14: Percentage of light and dark**

## DISCUSSION:

The light/dark box is also widely used for rodents as a model for screening anxiolytic or anxiogenic drugs, based on the innate 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<sup>17</sup>. 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 anxiolytic action<sup>18</sup>. The present study showed that AEMC could increase the time in the light area, suggesting again that AEMC possesses anxiolytic properties.

## CONCLUSION

In pharmacological screening method, the Cinnamum cassia bark 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 Cinnamomum cassia 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 off ever ,agitations and anxiety.

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