

Acute Toxicity Test and Behavioural Activity of Aqueous and Ethanol Dried Leaf Extracts of *Solenostemon monostachyus* In Mice

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The leaves of *Solenostemon monostachyus* have been traditionally used for various medicinal purposes, including CNS related ailments.

Objective: To evaluate the median lethal dose (LD₅₀), behavioural activity with possible mechanism(s) of action of aqueous dried leaf extract (AESM) and ethanol dried leaf extract (EESM) of *Solenostemon monostachyus*

Method: The aqueous and ethanol extracts were obtained using Soxhlet extraction and 80% ethanol respectively. The LD₅₀ of the extracts was determined orally (*p.o.*) and intraperitoneally (*i.p.*). The effects of the AESM and EESM (200, 400, 800 mg/kg, *p.o.*, n=6) on novelty-induced behaviours (NIB) were evaluated using the open field test. Possible mechanism(s) were evaluated using antagonists: flumazenil, naloxone and cyproheptadine at 2 mg/kg each, atropine and yohimbine at 1 mg/kg each.

Results: The LD₅₀ values obtained for AESM and EESM were 3808 and 2154 mg/kg *p.o.* respectively, while *i.p.*, values were 490 and 693 mg/kg respectively. AESM and EESM (200, 400, 800 mg/kg, *p.o.*) significantly ($p < 0.05$) reduced the NIB. The probable pathways could be majorly facilitated through adrenergic, and GABAergic pathways, though the serotonergic, opioidergic and muscarinic pathways may be implicated. These pathways, except the muscarinic pathway may be implicated in the action of EESM.

Conclusion: The aqueous and ethanol dried leaf extracts of *S. monostachyus* showed slight and moderate toxicity respectively, suppressed CNS which could be mediated mainly through antagonism of adrenergic and augmentation of GABAergic neurotransmission, while cholinergic (except EESM), opioidergic and serotonergic pathways may be involved.

Keywords: *Solenostemon monostachyus*, Plant extracts, Median lethal dose, Behavioural mechanism

INTRODUCTION

The use of medicinal plants to treat a wide range of ailments has expanded in developing countries (Theophine *et al.*, 2014). The increase can be attributed to a number of factors, including reports of health-promoting effects of natural products, particularly extracts and products derived from plants, as well as the discovery of new bioactive compounds that can be used as lead compounds in the discovery and development of useful drugs

(Vuorelaa *et al.*, 2004; Calixto, 2019). Herbal medications are claimed to be safer and have fewer adverse effects than manufactured pharmaceuticals (Mohd *et al.*, 2019). Consequently, many developing countries, including Nigeria, have conducted studies on their use. There are a variety of herbal remedies for nourishment and treatment in every local community. People's use of these herbs, and how they consume them, are influenced by demographic considerations (Vabo and Hansen, 2014), as well as sociological and cultural evolution (Risvik *et al.*,

2006). *Solenostemon monostachyus* sp. Beauv (Lamiaceae) is a valuable West and Central African herb. It is a valuable herb that thrives in both anthropogenic and rocky savannah environments. The leaves of *S. monostachyus* have been used for numerous therapeutic reasons as a decoction (extraction of plant elements by boiling in water). The plant has traditionally been used to prevent miscarriage and ease childbirth (Djah and Danho *et al.*, 2011). The leaf decoction also has diuretic properties (Koffi *et al.*, 2006). The aerial component (Ekundayo and Ezeogu, 2006) and the plant's leaf have both been shown to have antibacterial action in previous studies (Baba and Onanuga, 2011). The plant's aerial portions are used in a variety of decoctions by the Ibibios of Nigeria's Niger Delta to treat stomach ulcers, fever/malaria (Ajibesin *et al.*, 2008; Adebayo and Krettli, 2011), haemorrhoids, and other inflammatory disorders. It is used to treat measles and dizziness, among other things (NNMDA, 2006). The leaves of *S. monostachyus* are used to treat a number of diseases, including convulsions and fever in children. The leaves are also used to cure dysmenorrhoea, haematuria, infertility in women, rheumatism and foot infections (Lemmens, 2004). Extracts from *S. monostachyus* have been shown to have larvicidal activities on Anopheles mosquitoes (Chukwura and Iheukwumere, 2013). Ethanol extract of *S. monostachyus* produced a dose-dependent anticonvulsant activity against pentylenetetrazole and strychnine induced seizure (Oden-Onu, 1996)

The ethanol extract and fractions of the aerial parts exhibited anti-ulcer property in rat (Louis *et al.*,

METHODOLOGY

Plant collection, identification, authentication and preparation

The plant *S. monostachyus* was collected around drainages of the Faculty of Agriculture in Obafemi Awolowo University, Ile-Ife, Nigeria. It was initially identified and authenticated by Mr. I.I. Ogunlowo, the herbarium officer of Faculty of Pharmacy, OAU, Ile-Ife and confirmed by Mr. G.A. Ademoriyo, Botany Department, Faculty of Science, OAU, Ile-Ife and herbarium specimen number IFE-17638 was issued.

The fresh leaves were detached from the stalk and air dried under shade for about two weeks. The *S. monostachyus* dried leaves were powdered with laboratory mill. The powdered leaf (500 g) was weighed and the aqueous extraction done using soxhlet extraction method for about 48 h. The extract

(2015). Hydroethanolic leaf extract reduced high blood pressure (Kpahe, 2012). Methanolic extract has significant anti-oxidant activity (Tebekeme and Diepreye, 2012). Leaf extract and fractions have antiplasmodial activity. Antipyretic efficacy was also found in chloroform and aqueous leaf extracts against 2,4-dinitrophenol and yeast-induced pyrexia (Okokon *et al.*, 2015). The aerial parts ethanolic extract has anti-inflammatory and analgesic effects (Okokon *et al.*, 2016). Hydroethanolic leaf extract has a prolactin reducing activity. (Omoloye *et al.*, 2015). The ethanol leaf extract has hepatoprotective activity (Asanga *et al.*, 2015). *S. monostachyus* crude plant phytochemical screening revealed the presence of anthraquinone, alkaloids, flavonoids, saponin, tannins, and cardenolides. Water, proteins, lipids, calcium, phosphate (Buisson *et al.*, 1965), essential oil (Mve-Mba *et al.*, 1994), diterpenoids (Toshio *et al.*, 1980), coumarin, and polyphenol (Datte *et al.*, 2010; N'guessan *et al.*, 2011) were discovered in phytochemical studies on *S. monostachyus* leaves. The leaf essential oil of *S. monostachyus* has been reported to contain; β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol and (E,E)- α -farnesene (Mvé-Mba *et al.*, 1994). The plant has been reported to be useful in the treatment of convulsion, anxiety and panic attacks. However, the comprehensive scientific basis for the central nervous system (CNS) activities has not been evaluated. Hence, this study evaluated the CNS activities and the mechanism(s) of action and also determined the acute toxicity profile of the aqueous and ethanol leaf extracts of the plant.

was then dried in an oven maintained at 40 °C before being kept in the desiccator until use.

For the ethanol extraction, the powdered leaf (500 g) was macerated with 3 L of 80 % ethanol for 72 h. The mixture was filtered and the filtrate concentrated to dryness *in vacuo* using a rotary evaporator at 40 °C. The extract was subsequently kept in the desiccator until use. The percentage yield of AESM was 3.55 % while that of EESM was 2.63 %.

Laboratory materials

Drugs

Diazepam (Roche, Basel, Switzerland), naloxone, flumazenil (Sigma Chemical Co, St Louis, USA), yohimbine (Sigma Chemical Co, St Louis, USA), atropine (Paulo, Lagos, Nigeria), cyproheptadine (Therapeutic Laboratories, Lagos, Nigeria).

Laboratory animals

Adult male and female albino mice (18 - 25 g) obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife were used for the study. They were supplied with food and water *ad libitum*. Extracts and test drugs were administered orally (*p.o.*), while antagonists were given intraperitoneally (*i.p.*). The animal experiments were carried out according to the protocol approved with the number: PHP14/15/H/0209 by the Postgraduate College on behalf of the Obafemi Awolowo University Research Committee on 20th October, 2016.

Acute toxicity studies

The method used was described by Lorke (1983). In the first phase, three increasing doses (10, 100 and 1000 mg/kg) of the extracts were administered intraperitoneally and orally to three different groups of mice (n=3). In the second phase, 4 dose levels 400, 600, 800 and 1000 mg/kg of the aqueous and ethanol extracts were administered intraperitoneally to 4 groups of mice (n=1) and four dose levels (1000, 1600, 2900, 5000 mg/kg) of both extracts were administered orally to 4 groups of mice (n=1). The animals were observed for immediate effects of the extracts up to 30 minutes and the mortality within 24 hours of treatment was recorded. The median lethal dose (LD₅₀) value of each extract was calculated as: $LD_{50} = (A \times B)^{1/2}$ where A is maximum dose that resulted to 0 % death and B is the minimum dose that resulted to 100 % death.

The choice of route of administration for behavioural study

The oral route was used for the study to replicate the folkloric route of administration. The working doses used were 200, 400 and 800 mg/kg, *p.o.* which were lower than half of the LD₅₀ values for both the aqueous and ethanol extracts estimated to be 3808 and 2154 mg/kg, *p.o.* respectively.

Novelty-induced behaviours (NIB): Rearing and line crossing.

Open field model described (Ajayi and Ukponmwan, 1994) was used with minor modification to test the

novelty-induced behavioural activities (rearing and line crossing). There were two sets of five groups of mice (n=6 per group). For the first set, group 1 was administered with the vehicle (0.1 ml/10 g *p.o.* normal saline) to serve as negative control, groups 2 - 4 were administered with different doses (200, 400 and 800 mg/kg, *p.o.*) of the aqueous extract and group 5 received diazepam (1 mg, *p.o.*) to serve as positive control. For the second set, group 1 was administered with the vehicle (0.1 ml/10 g *p.o.* 5 % Tween 80) to serve as negative control, groups 2 - 4 were administered with different doses (200, 400 and 800 mg/kg, *p.o.*) of the ethanol extract and group 5 received diazepam (1 mg, *p.o.*) to serve as positive control. All the mice in each of the groups (1-5) were pre-treated with the respective extract and drug for 1 h prior to test. Each animal was placed inside the observation cage and assessed for rearing (number of times the animal stood on its hind legs or leaned its fore limbs against the wall of the observation cage or raise them freely in the air) and line crossing (number of squares crossed on the observation cage floor with all the four limbs), for 20 min.

Influence of antagonists on the effect of AESM and EESM on NIB

For each of the extracts, there were 10 groups of mice (n= 6 per group). Groups 1-5 were administered with yohimbine (1 mg/kg *i.p.*), atropine (1 mg/kg *i.p.*), naloxone (2 mg/kg, *i.p.*), cyproheptadine (2 mg/kg *i.p.*) and flumazenil (2 mg/kg *i.p.*). Each set of groups 6-10 was administered with 800 mg/kg, *p.o.* aqueous and ethanol extracts respectively after 30 min pre-treatment with each of the antagonists except for flumazenil and naloxone that were 15 min pre-treated. Each animal was placed inside the observation cage and observed for rearing and line crossing for 20 min (Oyemitan *et al.*, 2008; Olayiwola *et al.*, 2013).

Statistical analysis

The results were expressed as Mean \pm SEM and analysed using one-way analysis of variance (ANOVA) followed by post hoc test using Dunnett's comparison test and Student-Newman-Keuls test. The level of significance was set at 95% confidence interval at $p < 0.05$ for all treatments carried out compared to control groups. Graph pad prism, version 5.0 (UK) was used.

RESULTS AND DISCUSSION**Acute toxicity test****Table 1: Oral acute toxicity effect of aqueous and ethanol extracts of *Solenostemon monostachyus* in mice**

Dose (mg/kg)	Death patterns after 24hours	
	Aqueous extract	Ethanol extract
Phase 1 (n= 3)		
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
Phase 2 (n= 1)		
1000	0/1	0/1
1600	0/1	0/1
2900	0/1	1/1
5000	1/1	1/1
LD₅₀	$\sqrt{(2900 \times 5000)} = 3808 \text{ mg/kg}$	$\sqrt{(1600 \times 2900)} = 2154 \text{ mg/kg}$

$$LD_{50} = \sqrt{A \times B}$$

Where A is the maximum dose that resulted into 0% death and B is the minimum dose that resulted into 100% death.

Table 2: Intraperitoneal acute toxicity effect of aqueous and ethanol extracts of *Solenostemon monostachyus* in mice

Dose (mg/kg)	Death patterns after 24hours	
	Aqueous extract	Ethanol extract
Phase 1 (n= 3)		
10	0/3	0/3
100	0/3	0/3
1000	3/3	1/3
Phase 2 (n= 1)		
400	0/1	0/1
600	1/1	0/1
800	1/1	1/1
1000	1/1	1/1
LD₅₀	$\sqrt{(400 \times 600)} = 490 \text{ mg/kg}$	$\sqrt{(600 \times 800)} = 693 \text{ mg/kg}$

$$LD_{50} = \sqrt{A \times B}$$

Where A is the maximum dose that resulted into 0% death and B is the minimum dose that resulted into 100% death.

Effect of AESM and EESM on novelty-induced behaviours

The AESM at 200, 400 and 800 mg/kg, *p.o.* and the positive control (diazepam 1 mg/kg, *p.o.*) had significant effect [$p < 0.01$; $F_{(4, 25)} = 9.844$] compared to the vehicle (normal saline). This result is presented in Figure 1a. At 200 400 and 800 mg/kg, *p.o.*, AESM and diazepam 1 mg/kg, *p.o.* caused a significant reduction in the number of lines crossed [$p < 0.05 - 0.01$; $F_{(4, 25)} = 10.929$] compared to the vehicle (Figure 1b.)

The EESM at 200, 400 and 800 mg/kg, *p.o.* and diazepam 1 mg/kg *p.o.* reduced rearing in mice significantly [$p < 0.01$; $F_{(4, 25)} = 11.527$] compared to the vehicle (5% Tween 80). This result is presented in Figure 2a. At 200 400 and 800 mg/kg, *p.o.*, EESM and diazepam 1 mg/kg, *p.o.* caused a significant reduction in the number of lines crossed compared to the vehicle [$p < 0.01$; $F_{(4, 25)} = 12.550$] (Figure 2b.)

Influence of different antagonists on the effect of AESM on novelty-induced behaviours: rearing and line crossing.

Aqueous dried leaf extract of *S. monostachyus* at 800 mg/kg, *p.o.* and each of the antagonists (yohimbine 1 mg/kg, *i.p.*, flumazenil 2 mg/kg, *i.p.*, naloxone 2 mg/kg, *i.p.* and cyproheptadine 2 mg/kg, *i.p.*) when administered singly, significantly reduced the number of rearing [$p < 0.05 - 0.01$; $F_{(6, 35)} = 24.611$] compared to the vehicle (normal saline) but atropine 1 mg/kg, *i.p.*, did not significantly reduce rearing when compared to the vehicle. Similarly, co-administration of each of the antagonists with AESM 800 mg/kg, *p.o.* showed significant difference in rearing [$p < 0.05 - 0.01$; $F_{(5, 30)} = 70.888$] compared to AESM. This result is presented in Figure 3a and b.

The AESM at 800 mg/kg, *p.o.* and each of the antagonists (flumazenil 2 mg/kg, *i.p.*, naloxone 2 mg/kg, *i.p.*) when administered singly, significantly reduced the number of lines crossed [$p < 0.05 - 0.01$; $F_{(6, 35)} = 21.664$] in the open field compared to the vehicle but atropine 1 mg/kg, *i.p.*, yohimbine 1 mg/kg, *i.p.*, and cyproheptadine 2 mg/kg, *i.p.*, did not have any significant effect on line crossing compared to the vehicle when used singly. The co-administration of each of the antagonists (except flumazenil 2 mg/kg, *i.p.*) with AESM 800 mg/kg, *p.o.* showed significant difference in the number of lines crossed [$p < 0.05 - 0.01$; $F_{(5, 30)} = 25.775$] in the open field compared to AESM (Figure 4a and b.)

Ethanol dried leaf extract of *S. monostachyus* (EESM 800 mg/kg, *p.o.*) and each of the antagonists (except yohimbine 1 mg/kg, *i.p.*, and flumazenil 2 mg/kg, *i.p.*) when administered singly, significantly [$p < 0.01$; $F_{(6, 35)} = 25.070$] decreased rearing behaviour in mice compared to the vehicle (5% Tween 80). Co-administration of each of the antagonists (with the exception of atropine 1 mg/kg, *i.p.*) and EESM 800 mg/kg, *p.o.* showed significant reduction in rearing behaviour [$p < 0.01$; $F_{(5, 30)} = 20.711$] in mice relative to EESM (Figure 5a and b.)

The EESM, cyproheptadine 2 mg/kg, *i.p.*, and naloxone 2 mg/kg, *i.p.* when administered singly, each significantly decreased the number of lines crossed [$p < 0.05 - 0.01$; $F_{(6, 35)} = 21.950$] in the open field compared to the vehicle (5% Tween 80). The co-administrations of each of the antagonists (except atropine 1 mg/kg, *i.p.*, and yohimbine 1 mg/kg, *i.p.*) with EESM showed a significant reduction in the number of lines crossed [$p < 0.01$; $F_{(5, 30)} = 19.648$] in the open field compared to EESM (Figure 6a and b.)

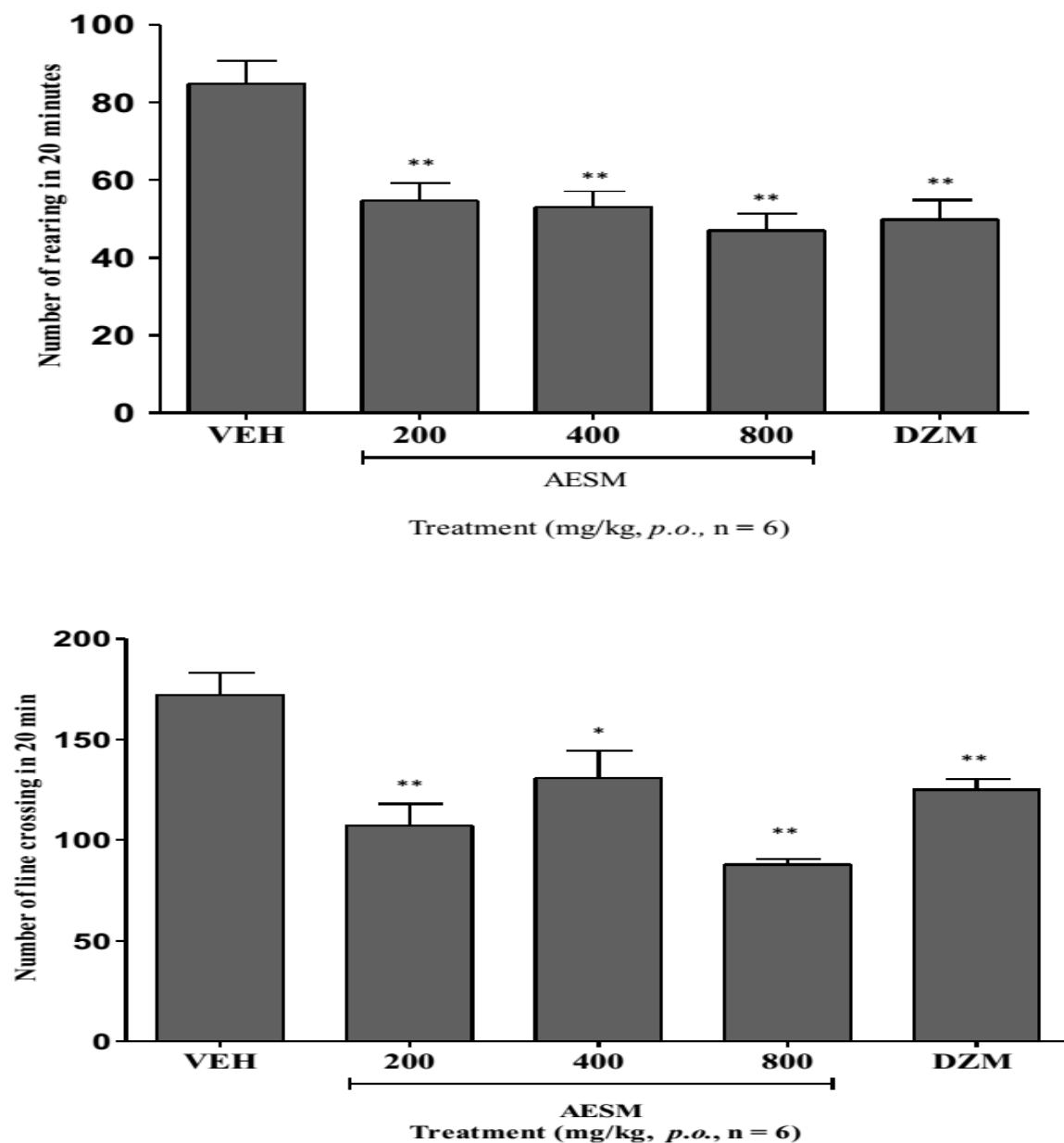


Figure 1 a and b: Effect of AESM on novelty-induced rearing (a) and line crossing (b)

Bars represent mean values with error bars (n = 6). VEH, AESM and DZM represent vehicle (normal saline), aqueous dried leaf extract of *S. monostachyus* and diazepam respectively.

* $p < 0.05 - 0.01$ statistically lower than vehicle (ANOVA, Dunnett's comparison test).

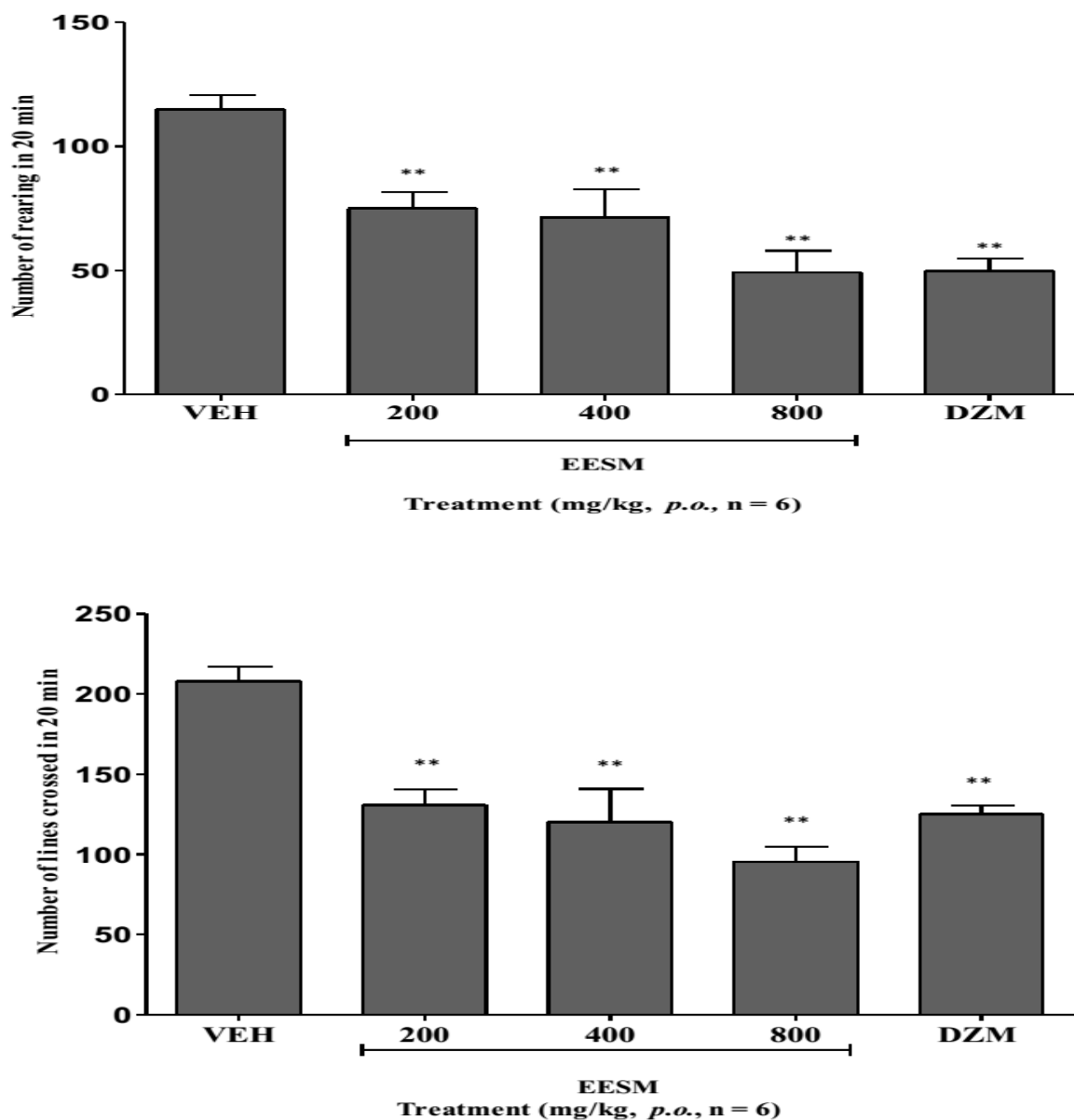


Figure 2 a and b: Effect of EESM on novelty-induced rearing (a) and line crossing (b)
Bars represent mean values with error bars (n = 6). VEH, EESM and DZM represent vehicle (5% Tween 80), ethanol dried leaf extract of *S. monostachyus* and diazepam respectively.
*p < 0.05 statistically lower than vehicle (ANOVA, Dunnett's comparison test).

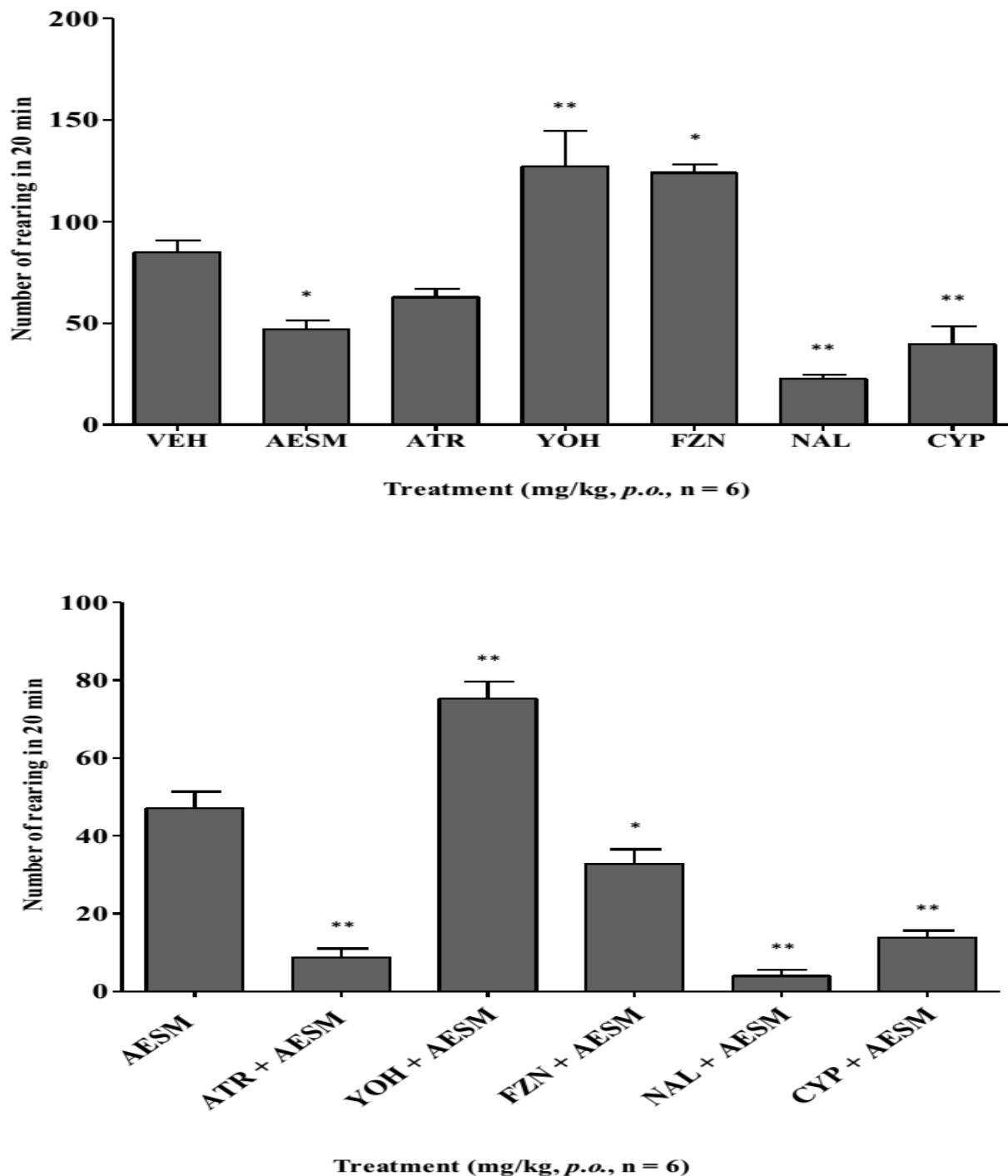


Figure 3 a and b: Sole effect of each of the antagonists and AESM on novelty-induced rearing (a) and Influence of antagonists on the effect of AESM on novelty-induced rearing (b)

Bars represent mean values with standard error of mean \pm SEM. VEH, AESM, ATR, YOH, FZN, NAL and CYP represent vehicle (normal saline), aqueous dried leaf extract of *S. monostachyus* (800 mg/kg), atropine (1 mg/kg), yohimbine (1 mg/kg), flumazenil (2 mg/kg), naloxone (2 mg/kg) and cyproheptadine (2 mg/kg) respectively. * $p < 0.05$ - 0.01 statistically significant compared to the vehicle (ANOVA, Student-Newman-Keuls test).

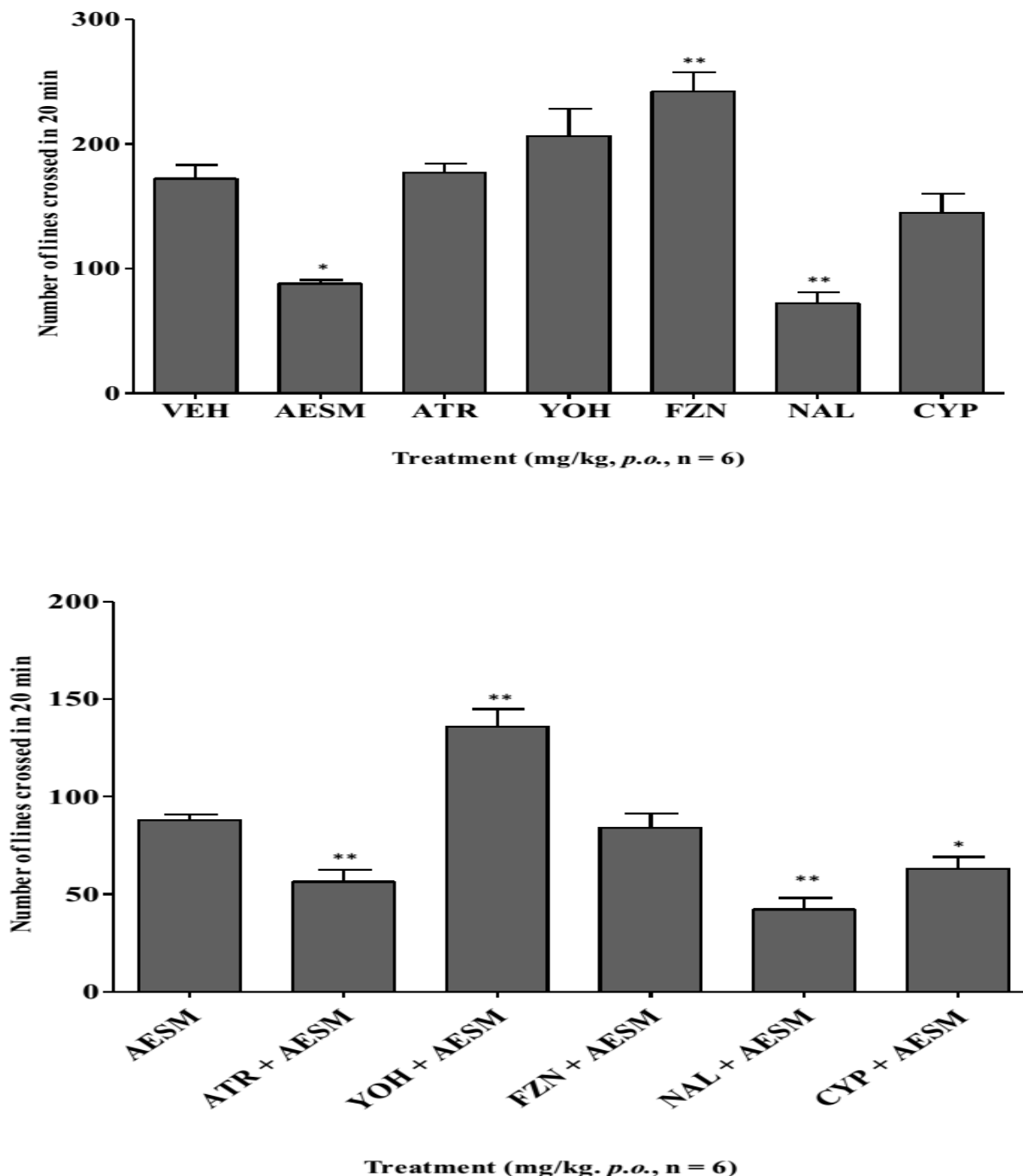


Figure 4 a and b: Sole effect of each of the antagonists and AESM on novelty-induced line crossing (a) and Influence of antagonists on the effect of AESM on novelty-induced line crossing (b)

Bars represent mean values with standard error of mean \pm SEM. VEH, AESM, ATR, YOH, FZN, NAL and CYP represent vehicle (normal saline), aqueous dried leaf extract of *S. monostachyus* (800 mg/kg), atropine (1 mg/kg), yohimbine (1 mg/kg), flumazenil (2 mg/kg), naloxone (2 mg/kg) and cyproheptadine (2 mg/kg) respectively. * $p < 0.05$ - 0.01 statistically significant compared to the vehicle (ANOVA, Student-Newman-Keuls test).

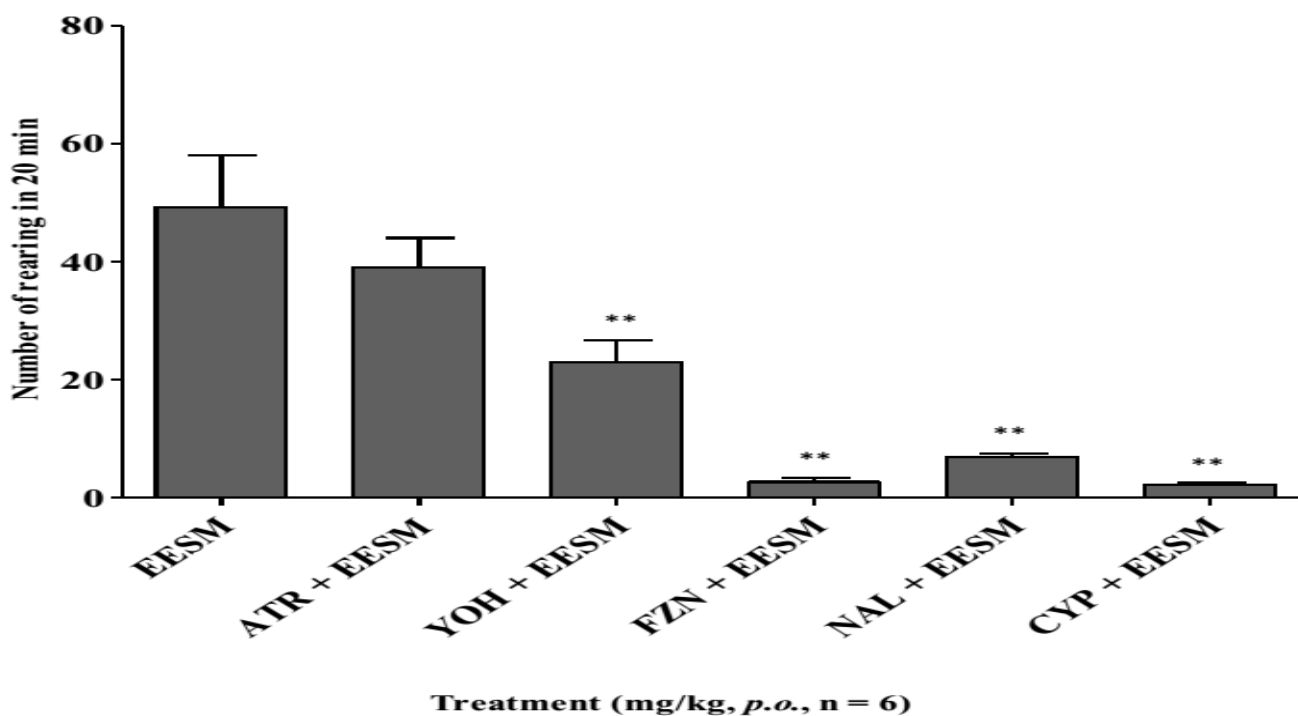
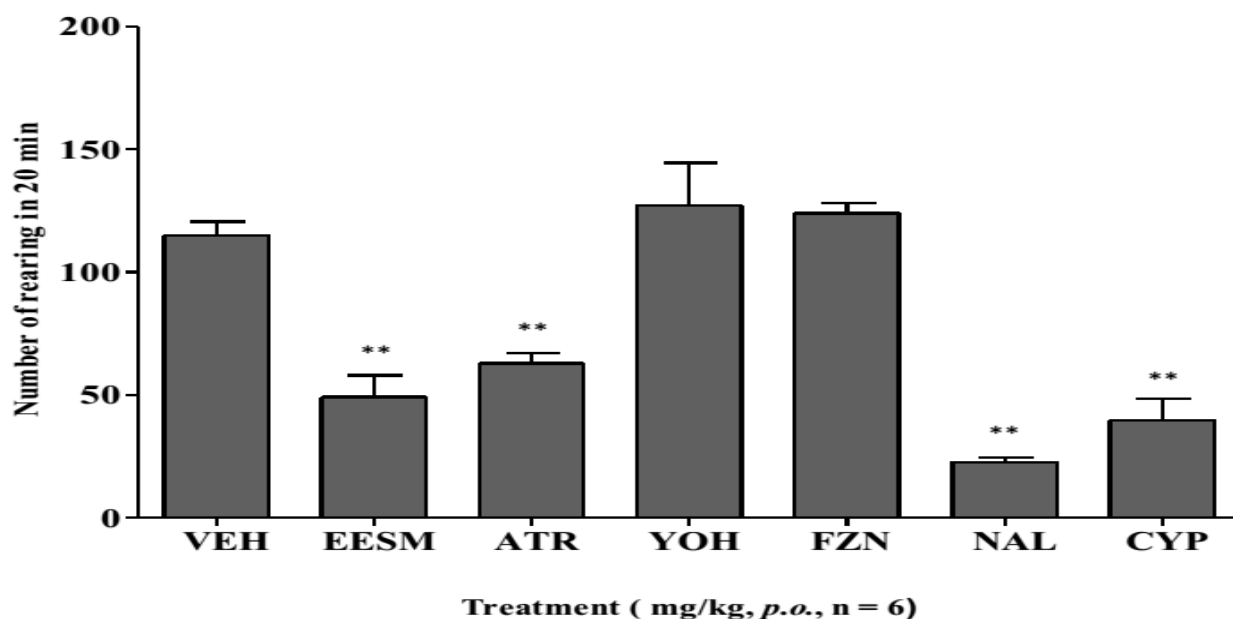


Figure 5 a and b: Sole effect of each of the antagonists and EESM on novelty-induced rearing (a) and Influence of antagonists on the effect of EESM on novelty-induced rearing (b)

Bars represent mean values with standard error of mean \pm SEM. VEH, EESM, ATR, YOH, FZN, NAL and CYP represent vehicle (5% Tween 80), ethanol dried leaf extract of *S. monostachyus* (800 mg/kg), atropine (1 mg/kg), yohimbine (1 mg/kg), flumazenil (2 mg/kg), naloxone (2 mg/kg) and cyproheptadine (2 mg/kg) respectively. * $p < 0.05$ - 0.01 statistically significant compared to the vehicle (ANOVA, Student-Newman-Keuls test).

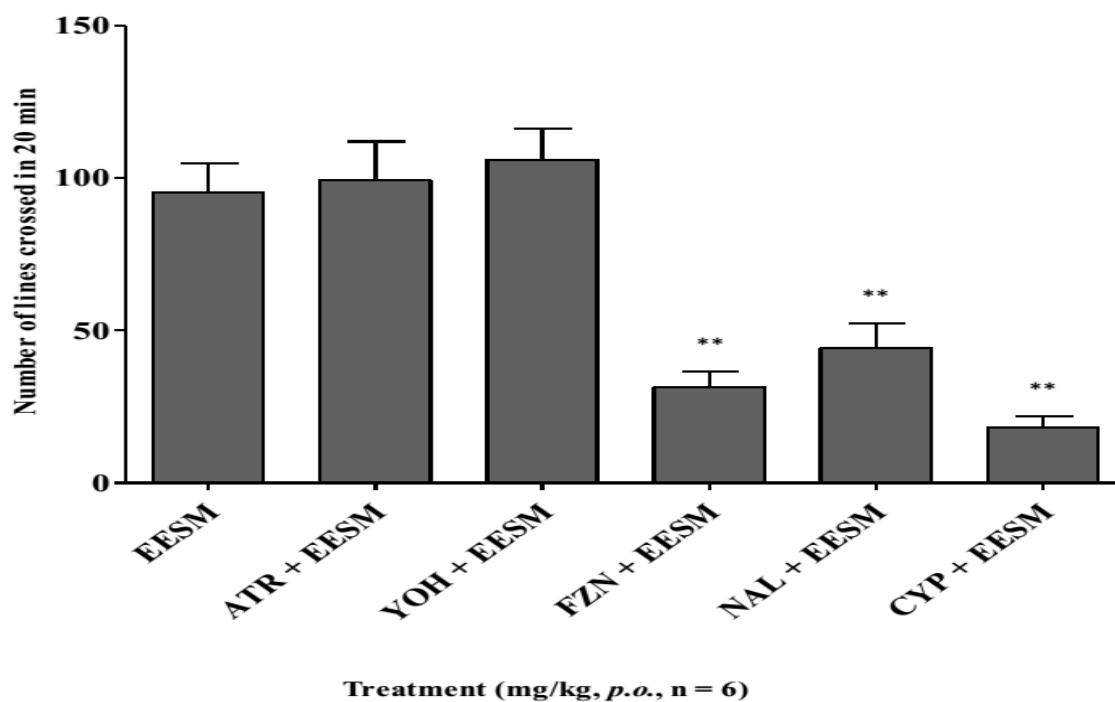
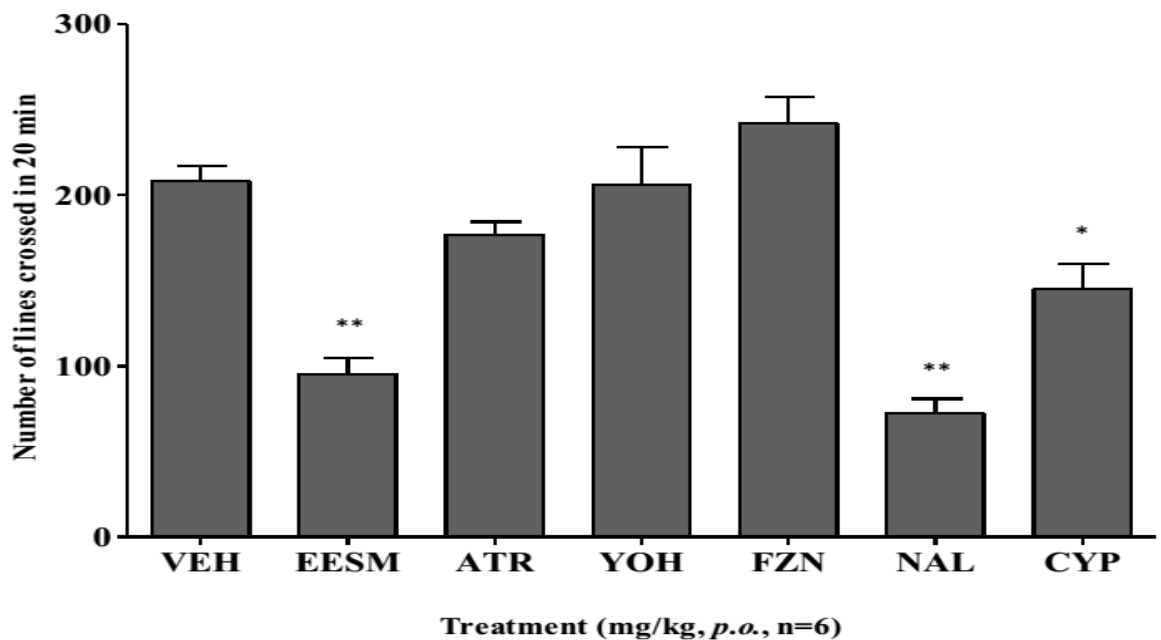


Figure 6 a and b: Sole effect of each of the antagonists and EESM on novelty-induced line crossing (a) and Influence of antagonists on the effect of EESM on novelty-induced line crossing (b)

Bars represent mean values with standard error of mean \pm SEM. VEH, EESM, ATR, YOH, FZN, NAL and CYP represent vehicle (5% Tween 80), ethanol dried leaf extract of *S. monostachyus* (800 mg/kg), atropine (1 mg/kg), yohimbine (1 mg/kg), flumazenil (2 mg/kg), naloxone (2 mg/kg) and cyproheptadine (2 mg/kg) respectively. * $p < 0.05$ - 0.01 statistically significant compared to the vehicle (ANOVA, Student-Newman-Keuls test).

DISCUSSION

The major effect of the extracts observed was depression of the CNS. The initial stage in determining the toxicity profile of unknown substances, such as medicinal plant extracts, isolates, natural products, and related chemicals, is to do acute toxicity testing. The LD₅₀ is an index of acute toxicity that provides preliminary information on acute toxicity with regards to the chemical, dose ranges, or route of administration (Lorke, 1983). From the acute toxicity study, the LD₅₀ of AESM was found to be 490 mg/kg, *i.p.*, 3808 mg/kg, *p.o.*; 693 mg/kg, *i.p.*, 2154 mg/kg, *p.o.* for EESM. According to the Hodge and Sterner (2005) toxicity scale, AESM and EESM showed slight and moderate toxicity orally and intraperitoneally respectively. The result shows more toxicity for intraperitoneal administration than oral administration. This is in agreement with a former study which revealed that drugs administered through intraperitoneal route showed more toxicity than through oral route (Oyemitan *et al.*, 2009). Oral route of administration leads to lower absorption and lower bioavailability because of the acidic content and enzymes in the gastrointestinal tract (Gavhane *et al.*, 2012).

The result of the novelty-induced behaviours showed that all doses of AESM and EESM (200, 400, 800 mg/kg, *p.o.*) and diazepam 1 mg/kg, *p.o.* caused a significant decrease of NIB in mice compared to the vehicle. Rearing is part of the exploratory behaviours displayed by rodents (Abdel-Barry and Al-Hakeim, 2000). The extracts caused reduced exploratory behaviour in mice and therefore suggests a CNS depressant effect (Hellion-Ibarrola *et al.*, 1999). Assessment of the number of rearings in rodents can therefore be used in assessing test substances and extracts for both sedative property and central nervous system stimulation (Vogel, 2002). Novelty-induced rearing is regulated by multiple neurotransmitter systems (Karczmar, 1993) which include acetylcholine, dopamine, serotonin, gamma-amino butyric acid, opioid and noradrenaline (Karczmar, 1993; Garret *et al.*, 2003) hence, these pathways may be implicated in the effects of the extracts. Line crossing is mediated through dopaminergic pathway (Rang *et al.*, 1999), hence suggesting a possible reduction in dopamine level or activity by the extract. The reduction in motor activities of the animals given the extracts suggests either skeletal muscle relaxant (Adeyemi *et al.*, 2006) or depression of the CNS.

The AESM and EESM at 800 mg/kg *p.o.* displayed the highest inhibitory effect on all the NIB parameters assessed hence, it was used against each of the antagonists to evaluate their interaction at the receptor levels. The antagonists used include:

cyproheptadine (2 mg/kg, *i.p.*), atropine (1 mg/kg, *i.p.*), flumazenil (2 mg/kg, *i.p.*), Naloxone (2 mg/kg, *i.p.*) and yohimbine (1 mg/kg *i.p.*).

The co-administration of atropine, a muscarinic cholinergic receptor antagonist, with AESM significantly ($p < 0.01$) elicited a reduction in novelty-induced rearing and line crossing when compared against sole administration of AESM. This reveals that the AESM may have elicited its inhibitory effect through the blockade of the cholinergic transmission. However, this muscarinic receptor antagonism was not elicited by EESM, hence, this suggests that the pathway may not be involved in its NIB inhibition.

Naloxone which is a specific opiate antagonist when co-administered with AESM, significantly ($p < 0.01$) elicited a reduction in novelty-induced rearing and line crossing when compared against sole administration of AESM. This reveals that the naloxone did not elicit a reversal of the inhibitory effect of AESM. This same effect was observed when naloxone was co-administered with EESM. There was rather a further enhancement of the inhibitory effect, which may be suggestive of a synergistic effect.

The effect of yohimbine (α_2 -adrenoreceptor blocker that selectively facilitates noradrenaline release) on AESM showed significant ($p < 0.01$) reduction in the novelty-induced rearing and line crossing. This shows a blockade of the noradrenaline neurotransmission hence, suggesting the involvement of the α_2 -adrenergic pathway in eliciting its effect. The same effect was displayed by EESM on novelty-induced rearing when co-administered with yohimbine.

Flumazenil, (GABA_A antagonist) elicited significant ($p < 0.05$) inhibitory effect when co-administered with AESM on novelty-induced rearing. It can then be deduced that the mediation of the elicited inhibitory effect could be through enhancement of GABAergic neurotransmission by AESM. The GABAergic neurotransmission was not inhibited when flumazenil was co-administered with EESM, as a significant ($p < 0.01$) inhibition of both novelty-induced rearing and line crossing was elicited.

Furthermore, cyproheptadine (serotonin receptor antagonist) did not elicit a reversal of the inhibitory effect of AESM and EESM on novelty-induced rearing and line crossing, rather, there was a significant ($p < 0.01$) enhancement of the inhibitory effect by the blockade of the serotonergic transmission

The effect of the AESM on the neurotransmitter pathways explored in this study consistently showed inhibitory effects on the novelty-induced behaviours.

The effect therefore is suggested to be majorly facilitated through adrenergic, and GABAergic pathways, though the serotonergic, opioidergic and muscarinic pathways may be involved too. These pathways except the muscarinic pathway were shown to also be the possible mechanisms of action of EESM.

CONCLUSION

The aqueous and ethanol dried leaf extracts of *Solenostemon monostachyus* showed slight and moderate toxicity orally and intraperitoneally respectively. Both extracts displayed significant depressant activity on the central nervous system. The major mechanism(s) of neurobehavioural effects of both extracts could possibly be through inhibition of adrenergic, and /or augmentation of GABAergic pathways.

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