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Psychoneuropharmacological Properties of Eclipta Alba (LINN) in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Eclipta alba (Linn) (EA) has been found useful ethnomedicinally in the treatment of many medical conditions especially mental disorders.
Aims of the Study: This study aimed to evaluate the acute toxicity profile, behavioural activity, antipsychotic and mechanism of action of the Aqueous extract of *Eclipta alba* (AEA).

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Methodology: Using Lorke's method the LD_{50} of AEA was 1,264 mg/kg (i.p) and 3,807 mg/kg (oral) respectively was obtained, which shows that AEA was moderately toxic. The AEA was analysed using standard procedure: novelty-induced behaviour (NIB), through intraperitoneal route shows that AEA ($p < 0.0001$) exhibits depressant effects on the central nervous system (CNS), while the antipsychotic models (catalepsy, amphetamine-induced hyperlocomotion, swimming-induced grooming and apomorphine-induced climbing tests), ($p < 0.0001$) reveals that it possesses antipsychotic properties.
Conclusion: This study shows that the mechanism of action of AEA (antagonist model: haloperidol, atropine and cyproheptadine) ($p < 0.0001$) probably mediated via histaminergic, serotonergic and dopamine pathways, which provides scientific support for the ethno-medicinal use of the plant in the treatment of mental illness.

Keywords: Eclipta alba; novelty-induced behaviours; acute toxicity; aqueous extract; antipsychotic; amphetamine; catalepsy; climbing; mechanism; haloperidol' apomorphine.

1. INTRODUCTION

"The use of proven scientific herbal remedies was being encouraged by the world health organization (WHO) in health care delivery, especially among developing countries with advancement of traditional medicine while contributing conventional medical practice". Josephine Ozioma & Antoinette Nwamaka Chinwe, [1]. Scientific investigation must be conducted for herbal plants with claims of traditional medicinal use to improve health care delivery. *Eclipta alba* (*L*) (EA) belongs to the Asteraceae family, commonly known as false daisy in English with high value because of its ethnomedicinal significance (Azwanida, 2015). Studies have reported that EA contains a wide range of active phytochemicals which are found use in its ethnomedicinal actions these includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes and triterpenoids. The leaves contain stigmasterol, β-terthienyl- methanol, wedelolactone, dimethylwedelolactoneand demethylwedelolactone-7-glucoside" [2]. Similarly, it is a source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for the treatment of cirrhosis of the liver and infectious hepatitis [3] EA has been reported to be useful as catarrhal jaundice and for skin diseases [4]. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator [5]. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and is popularly used to enhance memory and learning [6]. The plant has a reputation as an antiaging agent in Ayurveda [7]. Externally it is used for inflammation [8] minor cuts, burns and fresh leaf juice is considered very effective in stopping bleeding [9]. Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections [9]. The AEA exhibited the most potent inhibitory activity against HIV-1 integrase (HIV-1 IN) Ali et al., [10] Jahan et al., [11]. Vedic Guard contains EA as a major ingredient in treatments of gastrointestinal disorders [12]. It was reported that taking the juice of EA with honey helps to prevent the onset of senility, and its oil as the best-medicated massage oil for rejuvenation therapies [13]. EA activities were found to associate with the central nervous system [14], hence it becomes imperative to further evaluate the acute toxicity and behavioural activity of the aqueous extract of *Eclipta alba* (AEA) and determine the behavioural and antipsychotic activities or the plant using standard models.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The fresh leaves of EA were collected from a private farm at Ile-Ife, Osun state, South-West Nigeria during raining season (June) in 2020. Botanical identification (FPI=2211) was performed and the voucher specimen was deposited at the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile Ife, Nigeria.

The plant was air-dried and grounded to powder. Then one thousand grams (1000g) of the powdered plant was macerated using distilled water for 24 hours. The resultant aqueous extract was then concentrated to obtain a black liquid which was dried over anhydrous sodium sulfate.

2.2 Drugs and Chemicals

The following drugs were used: Diazepam (Valium(R) Roche, Switzerland), normal saline

(Unique Pharm. Nig. Ltd.), haloperidol HCl, Apomorphine HCl, normal saline (Unique Pharm. Nig. Ltd.), and other reagents were of analytical grade.

2.3 Laboratory Animals

Adult male and female Swiss mice (VOM strain) 18–25g were obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. The animals were housed in cages at room temperature and maintained on standard animal pellets and water *ad libitum*.
The ethical clearance for this research was obtained through the Faculty Postgraduate Committee and all animal experiment was carried out in strict compliance with the National Institute of Health (NIH, 1996) as implemented by the OAU Research Committee. The research was conducted in a quiet laboratory.

2.4 General Experimental Design

2.4.1 Acute toxicity study

The acute toxicity of AEA was demonstrated in mice using the intraperitoneal (i.p.) and oral routes respectively according to Lorke's method (Lorke, 1983).The method involved an initial dose finding phase (first phase) using the dose levels of 10, 100, and 1000 mg/kg, using three mice per dose group. The animals were monitored for 24 hours for mortality and general behavior. The second phase involved three (3) dose levels obtained from the first phase following a standard table as described by Lorke's (1983).

Each animal was administered the required dose of AEA via the routes and then placed inside the Plexiglas cage for observation of immediate effects up to 30minutes and then 24 hours for the lethal effects (death). The LD_{50} of AEA was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death following the formula:

LD_{50}= √ maximum dose producing 0% death x minimum dose producing 100% death. (Lorke's 1983).

2.5 Experimental BehaviouralStudy

2.5.1 Effects of AEA on novelty-induced behaviours in mice

Animals were randomly selected into 5 groups (n=5). Group I was the negative control which received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with AEA at doses of 100, 200 and 400 mg/kg respectively, while the positive control group V, received the appropriate standard drug: Diazepam (DZP) (2 mg/kg, i.p.). All treatments were made by (i.p.) route.

The novelty induced behavioural (NIB) effects scores of rearing, grooming and locomotion were performed according to Onigbogi et al., (2000). Each mouse was placed inside Plexiglas's cage and observed for rearing (20 min) and locomotive activity (20 min) after 30 min of pre-treatment. The floor of the cage was divided into 16 equal squares and the number of squares crossed with all the fore and hind limbs was counted as locomotion, while rearing was the number of times the animal places its fore paws against the wall of the cage or in the air. Grooming involves nose and face washing and mouth cleaning. Before introducing each animal, the cage was cleaned with 5% alcohol to eliminate the possibility of any bias due to the odour that could have been left on the board by previous mouse.

2.6 Experimental Design for Antipsychotic

Animals were randomly selected into 5 groups (n=5). Group I serve as the negative control which received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups III–IV were treated with the AEA at doses of 100, 200 and 400 mg/kg respectively, while the positive control group II received the appropriate standard drug [Halopridol (HAL), Apomorhine (APO) and Amphetamine (AMP)]. All treatments were made by (i.p.) route.

2.7 Effect of the AEA on Experimental Psychosis

2.7.1 Effect of AEA on Catalepsy test

Group I was control while group II was standard control received HAL (2 mg/kg, i.p.) was used as the standard drug. Groups III –V received doses of AEA 100, 200 and 400 mg/kg with HAL. Each mouse was pre-treated for 30 min, later placed in the observation cage with its fore paws over a 3.5 cm bar and watched for the time it takes to remove its fore paws from the bar. This procedure was repeated at 30, 60 and 120min post-treatment. The intensity of catalepsy was measured by the duration of time the animal took

to remove both forelimbs from the bar to the floor of the observation cage [15,16].

2.7.2 Effect of AEA on apomorphine-induced climbing test

The method described by Davis[17] Seong et al.[18] was adopted. The following scoring system was employed: 0 = all paws on the cage floor; 1= two paws placed on the side of the cage; 2 = all paws off the floor; 3 animals climbed and remain on the wall. The scores achieved by individual animals were summed so that each animal obtained a final score between 0 and 6. Climbing behaviour assessment was for 2 min after 10, 20 and 30 min post APO injection (2 mg/kg, i.p.).

2.7.3 Swimming-induced grooming behaviour test

Grooming behaviour was induced in mice by a short period of swimming. Before short swimming in a chamber (8 cm high) filled with water (30^{o}C) for about 3 min, the animals were pre-treated following the standard protocol. Afterward, the animals were towel-dried for 20 seconds and immediately placed inside the observation cage and scored as follows: Presence of grooming =1; absence of grooming =0, for every 2 min and up to a total time of 20 min. The maximum score possible is 10 points" [19,20].

2.7.4 Amphetamine-induced hyperlocomotion test

Locomotor activity was assessed as described by Salahpour et al [21] Standard treatments were administered 30 min prior to amphetamine (2 mg/kg, i.p.). Spontaneous locomotor activity was measured immediately after mice were placed in the observation cage and counted the number of line crosses within 15 min. AMP (2 mg/kg, i.p.) was used as the positive control.

2.7.5 Assessment of mechanism of action of some antagonists (Haloperidol (HAL), Atropine (ATR), Cyproheptadine (CYP)) effects on AEA in NIB

The test was done to determine the effect of antagonists on AEA in NIB (locomotion, rearing, grooming) to explore the possible neurotransmitters or pathways through which the AEA exerts its effects and probable mechanism of action. Four groups (n=6), for each of the antagonists that were employed in this experiment. Group I was given normal saline (10µl/kg). Group II received vehicle and antagonist: HAL (2 mg/kg, i.p.), ATR (2 mg/kg, i.p.) and CYP (2 mg/kg, i.p.), group III received the vehicle and 400 mg/kg of AEA, (i.p.) and group IV received a dose of antagonist and 400 mg/kg of AEA, (i.p.). Each mouse was pre-treated with the antagonist for 15 minutes after which they were observed for NIB for 30minutes [22].

2.8 Statistical Analysis

The results were expressed as mean (SEM). All parametric tests were analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keul's test between the treated groups and control. The level of significance was set at a 95% confidence interval at $p < 0.05$. The statistical software used was GraphPad Instat3.0 and GraphPad Prism 5 (Copyright (c) 2007 GraphPad Software Inc.).

3. RESULTS

3.1 Acute Toxicity

The results of the acute toxicity study indicate that the LD_{50} of the AEA was calculated to be 1,264 mg/kg and 3,807 mg/kg for the intraperitoneal and oral routes, respectively. Table 1 and 2.

3.2 Behaviouralstudy

3.2.1 Novelty-induced behaviours

The AEA significantly decreased rearing [$F_{(6, 29)} = 12.46$, $p < 0.0001$)], grooming [$F_{(6, 29)} = 10.46$, $p< 0.0001$)] and locomotive activity [$F_{(6, 29)} = 17.13$, $p < 0.0001$)] compared to vehicle. Diazepam also demonstrated a similar effect. The AEA dose-dependently suppressed exploratory behaviour significantly (Figs 1- 3).

Table 1. Intraperitoneal route lethal dose toxicity profile of AEA in mice using Lorke's method (LD_{50})

Phase 1	Dose (mg/kg) (i.p.)	Mortality ratio
	10	0/3
	100	0/3

	1000	0/3
Phase 2	**Dose (mg/kg) (i.p.)**	**Mortality ratio**
	1600	1/1
	2900	1/1
	5000	1/1

LD_{50} (i.p) = √ (A × B)= √ (1600 × 1000)=1,264 mg/kg

Table 2. Oral route lethal dose toxicity profile of AEA in mice using Lorke's method (LD_{50})

Phase 1	**Dose (mg/kg) (i.p.)**	**Mortality ratio**
	10	0/3
	100	0/3
	1000	0/3
Phase 2	**Dose (mg/kg) (i.p.)**	**Mortality ratio**
	1600	0/1
	2900	0/1
	5000	1/1

LD_{50} (i.p) = √ (A × B)= √ (2,900 × 5000),= 3,807 mg/kg.

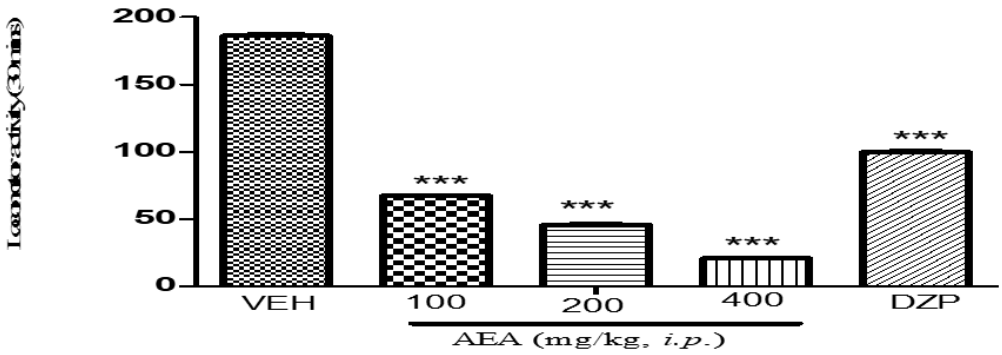

Fig. 1. Effect of AEA on Novelty-induced Locomotion in Mice

Bars represent mean values with a standard error of mean ± SEM

VEH, AEA and DZP represent vehicle (normal saline, 10 µl/kg, i.p.), aqueous extract of E.alba (100, 200 and 400 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) respectively, n=6

****p < 0.0001 statistically significant compared to vehicle.(ANOVA, Student-Newman-Keul's test)*

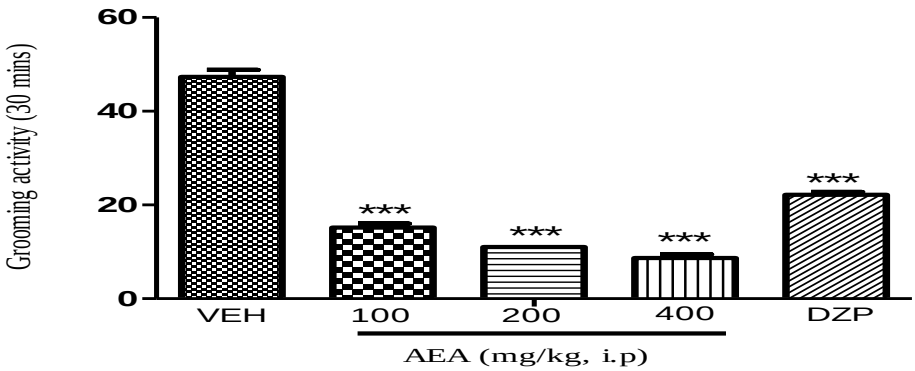

Fig. 2. Effect of AEA on novelty-induced grooming in mice

Bars represent mean values with a standard error of mean ± SEM

VEH, DZP and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), diazepam (2 mg/kg, i.p.) and aqueous extract of E.alba, respectively, n=6

****p < 0.0001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test)*

3.3 Antipsychotic Effects of AEA

3.3.1 Effect of the AEA on the catalepsy test in mice

The results obtained showed that AEA at doses of 100, 200 and 400 mg/kg, i.p. significantly [F(6,35)= 40 (p<0.0001)] increased the catalepsy effects induced by haloperidol (2 mg/kg, i.p.) compared to the vehicle-treated control group. The standard drug haloperidol (2 mg/kg, i.p.) significantly (p < 0.001 and p < 0.0001) increased the duration (of immobility) in the catalepsy test, compared to a vehicle-treated control group. Table 3.

3.3.2 Effect of the AEA on Apomorphine-induced climbing behaviour test in Mice

The results obtained showed that AEA at doses of 100, 200 and 400 mg/kg, i.p. significantly [$F_{(6, 35)}$ =90 p < 0.0001] inhibited climbing behaviour in the apomorphine-induced climbing test compared to the vehicle-treated control group. The standard drug apomorphine (2 mg/kg, i.p.), significantly (p < 0.0001) reduced the number of climbing compared to vehicle-treated control (Table4).

3.3.3 Effect of AEA on the Amphetamine–induced hyperlocomotion in Mice

The number of locomotion was significantly (p<0.0001) increased by Post-treatment with Amphetamine (2 mg/kg, i.p.) with reversal of inhibition occasioned by the AEA and significantly (p < 0.0001) decreased by standard diazepam (2 mg/kg, *i.p*.) when compared with the control. The hypermotility induced by amphetamine (2 mg/kg) was significantly [$F_{(6,35)}$ = 237 p< 0.001] decreased by AEA (100, 200 and 400 mg/kg, i.p.) when compared to amphetamine treated group (Fig. 4).

3.3.4 Effect of the AEA on swimming-induced grooming Test in mice

The AEA at doses of 100, 200 and 400 mg/kg, i.p. significantly [$F_{(6,29)}$=180 p< 0.0001) inhibited swimming-induced grooming behavior when compared to vehicle. Apomorphine (2 mg/kg. i.p.) significantly (p < 0.0001) reduced swimming-induced grooming behavior compared to vehicle. The result presented in Fig 5.

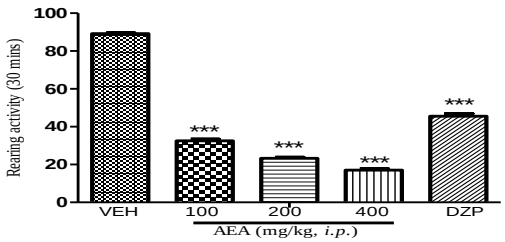

Fig. 3. Effect of AEA on novelty-induced rearing in mice

Bars represent mean values with a standard error of mean ± SEM. VEH, DZP and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), diazepam (2 mg/kg, i.p.), and aqueous extract of E.alba, respectively, n=6

****p < 0.0001 statistically significant compared to vehicle (ANOVA Student-Newman-Keul's test)*

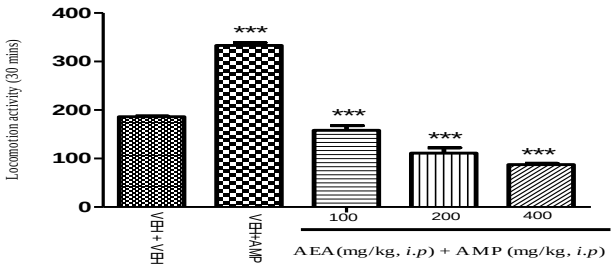

Fig. 4. Effect of AEA on the amphetamine–induced hyperlocomotion in Mice

Bars represent mean values with a standard error of mean ± SEM. VEH, DZP, AMP and AEA represent vehicle (normal saline 10 µl/kg, i.p.), Amphetamine (2 mg/kg, i.p.) and aqueous extract of E. alba, respectively, n=6

****p< 0.0001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test)*

Table 3. Effect of AEA on haloperidol-induced catalepsy in Mice

Treatment	15 Min	30 Min	45min	60 Min	90 Min	120 Min
VEH + VEH	0.17 ± 0.11	0.17 + 0.11**	0.08 ±0.08***	0.17 ± 0.11***	0.25 ± 0.11	0.25 ± 0.11*
VEH + HAL	0.42 ± 0.08	0.67 + 0.17	1.33 ± 0.17**	1.25 ±0.11***	0.92 ± 0.24	0.67 ± 0.17
AEA 100 + HAL	0.67 ± 0.17	1.75 ± 0.17	3.17 ± 0.2**	3.33 ± 0.17***	2.50 ± 0.26	2.1 7± 0.21***
AEA 200 + HAL	0.67 ± 0.17	1.75 ± 0.17	3.17 ± 0.2*	3.33 ± 0.17***	2.50 ± 0.26	2.1 7± 0.21***
AEA 400 + HAL	1.17 ±0.21*	2.83 ± 0.42 *	3.17 ± 0.2*	3.33 ± 0.17***	3.33 ± 0.17	3.17 ± 0.21***

HAL, VEH and AEA represent the Haloperidol (2 mg/kg, i.p.), vehicle (normal saline, 10µl/kg), n=6 and aqueous extract of E.alba, respectively, n=6. Catalepsy score express asmean ± SEM.
** = p< 0.05 compared with vehicle (normal saline)*
*** = p< 0.001 compared with vehicle (normal saline)*
**** = P< 0.0001 compared with vehicle (normal saline) using One-way analysis of variance (ANOVA) Student-Newman-Keul's test.*

Table 4. Effect of apomorphine-induce climbing on AEA in Mice

Treatment	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min
VEH	3.7 ± 0.2**	3.8 ± 0.2***	3.8 ± 0.2	3.5 ± 0.3***	3.8 ± 0.2***	3.8 ± 0.2*
VEH + APO	5.5 ± 0.2**	5.7 ± 0.2	6.0 ± 0.0	5.5 ± 0.2	5.5 ± 0.2	4.8 ± 0.2
AEA 100 + APO	2.8 ± 0.2***	2.3 ± 0.2***	2.8 ±0.3	3.3 ± 0.3***	3.7 ± 0.2***	3.7 ± 0.2*
AEA 200 + APO	2.5 ± 0.2***	2.2 ± 0.3***	2.0 ± 0.3	2.0 ± 0.3****	1.8 ± 0.3***	1.5 ± 0.2***
AEA 400+ APO	1.2 ± 0.2***	1.0 ± 0.3***	0.8 ± 0.3	0.8 ± 0.3***	0.3 ± 0.2***	0.2 ± 0.2***

APO, VEH and AEA represent the Apomorphine (2 mg/kg, i.p.), vehicle (normal saline) and aqueous extract of E.alba, respectively, n=6. The climbing score expresses as mean ± SEM
** = p< 0.05 compared with vehicle (normal saline)*
*** = p< 0.001 compared with vehicle (normal saline)*
**** = p< 0.0001 compared with vehicle (normal saline) using One-way analysis*

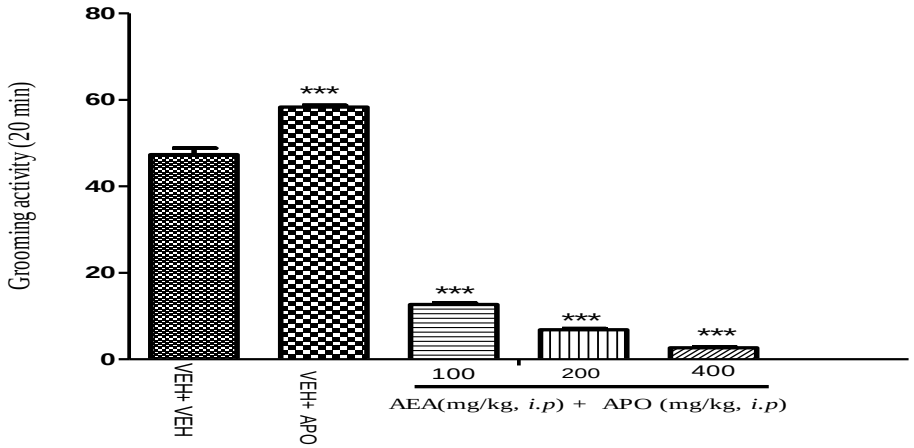

Fig. 5.Effect of apomorphine swimming-induced grooming activity on AEA in Mice
VEH, APO and AEA represent the vehicle (normal saline,10 µl/kg), Apomorphine (2 mg/kg, i.p.) and aqueous extract of E. alba, respectively, n=6. Score expressed as mean ± SEM
** = p< 0.05compared with vehicle (normal saline)*
*** = p< 0.001 compared with vehicle (normal saline)*
**** = p< 0.0001 compared with vehicle (normal saline) using One-way analysis of variance (ANOVA, Student-Newman-Keul's test)*

3.4 Mechanism of action of AEA using some Antagonists Model of AEA in Mice

3.4.1 Effect of Haloperidol on AEA activity on NIB in mice

The effect of haloperidol (2 mg/kg,.i.p.) on the inhibition of locomotion by AEA was investigated in mice. Haloperidol inhibited locomotion, grooming and rearing behaviour when compared with the control. The results obtained showed that the AEA (400 mg/kg, i.p.) significantly [F (4, 25) = 638, p < 0.0001)] reduced the number of locomotion, grooming and rearing, compared to the vehicle-treated as control group. The standard drug Haloperidol (1 mg/kg, i.p.) significantly (p < 0.0001) reduced the number of locomotion, grooming and rearing compared to the vehicle-treated control group. The result is presented in Figs 6-8.

3.4.2 Effect of Atropine on AEA activity on NIB in mice

The effect of Atropine (2 mg/kg, i.p.) on the inhibition of locomotion by AEA was investigated in mice. Atropine inhibited locomotion, grooming and rearing behaviour when compared with the control. The results obtained showed that the AEA (400 mg/kg, i.p.) significantly [$F_{(4, 25)}$ = 601.9, p < 0.0001] p < 0.0001) reduced the number of locomotion, grooming and rearing compared to the vehicle-treated control group. The standard drug Atropine (2 mg/kg, i.p.) significantly (p < 0.0001) reduced the number of locomotion, grooming and rearing compared to the vehicle-treated control (Figs 9-11).

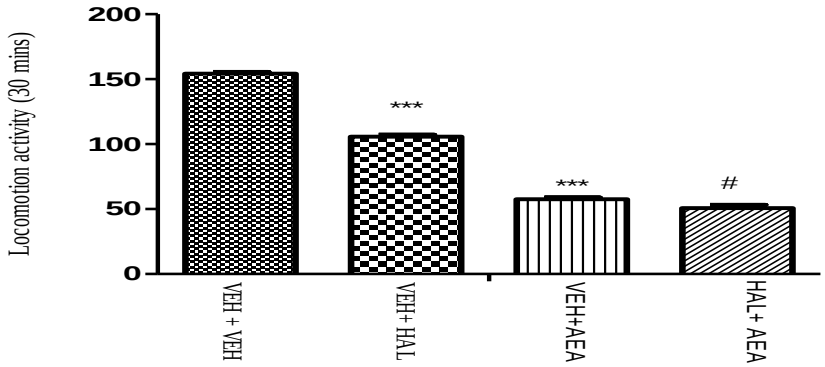

Fig. 6. Effect of haloperidol on AEA activity on NIB locomotion in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, DZP, HAL and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), haloperidol (2 mg/kg) and aqueous extract of E. alba, respectively, n=6.
****p < 0.01 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test). # p < 0.05 statistically significant compared to Veh+AEA*

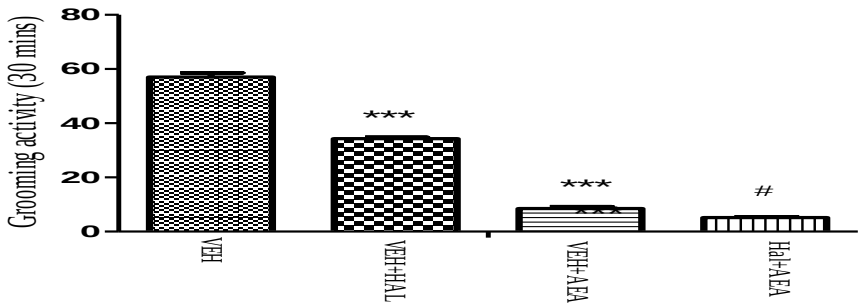

Fig. 7. Effect of haloperidol on AEA activity on NIB grooming in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, DZP, HAL and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), haloperidol (2 mg/kg) and aqueous extract of E.alba, respectively, n=6.
****p < 0.01 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test). # p < 0.05 statistically significant compared to Veh+AEA*

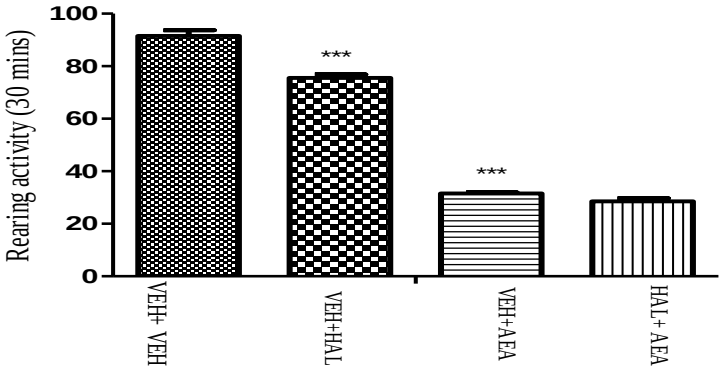

Fig. 8. Effect of haloperidol on AEA activity on NIB rearing in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, DZP, HAL and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), haloperidol (2 mg/kg) and aqueous extract of E. alba, respectively, n=6
****p < 0.01 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test)*

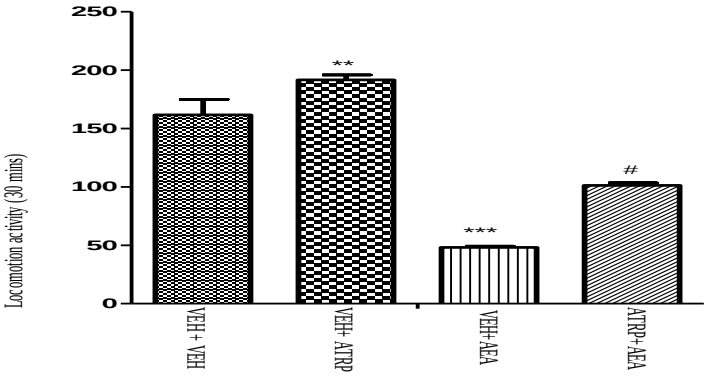

Fig. 9. Effect of atropine on AEA activity on NIB locomotion in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, ATRP and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), Atropine (2 mg/kg. i.p.) and aqueous extract of E.alba, respectively, n=6.
***p < 0. 01 when compared with vehicle (ANOVA, Student-Newman-Keul's test).*
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).*
###p < 0.001 statistically significant compared to Veh+AEA

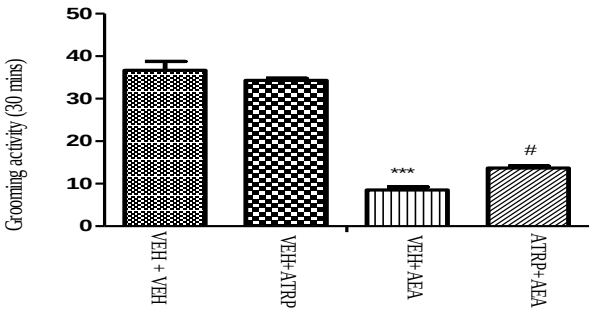

Fig. 10. Effect of Atropine on AEA activity on NIB grooming in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, ATRP and EEA represent vehicle (normal saline, 10 µl/kg, i.p.), Atropine (2 mg/kg. i.p.) and aqueous extract of E. alba, respectively, n=6
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).*
##p < 0.01 statistically significant compared to Veh+AEA

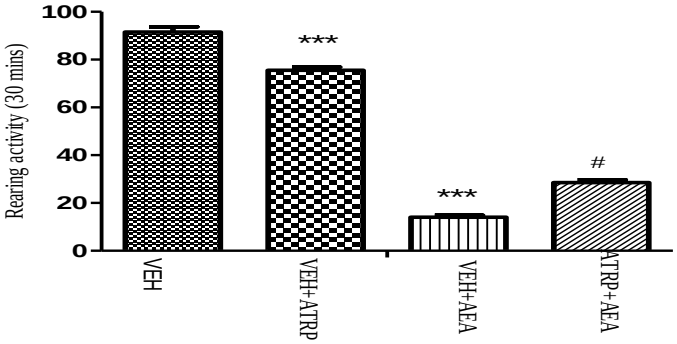

Fig. 11. Effect of Atropine on AEA activity on NIB rearing in mice

Bars represent mean values with a standard error of mean ± SEM
VEH, ATRP and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), Atropine (2 mg/kg. i.p.) and aqueous extract of E. alba, respectively, n=6
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).*
###p < 0.001 statistically significant compared to Veh+AEA

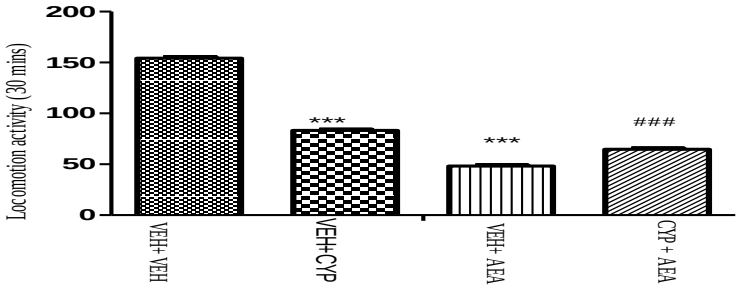

Fig. 12. Effect of Cyproheptadine on AEA activity on NIB locomotion in mice

Bars represent mean values with a standard error of mean ± SEM
VEH, CYPRO, and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), Cyproheptadine (2 mg/kg, i.p.) and aqueous extract of E. alba, respectively, n=6
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test)*
###p < 0.001 statistically significant compared to Veh+AEA

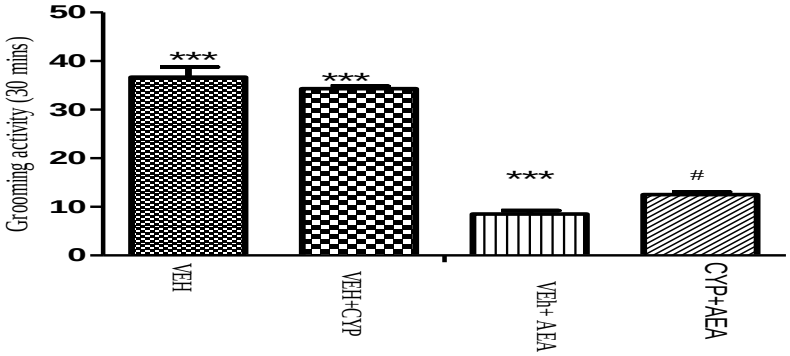

Fig. 13. Effect of Cyproheptadine on AEA activity on NIB grooming in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, CYPRO, and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), cyproheptadine (2 mg/kg, i.p.) and aqueous extract of E. alba, respectively, n=6
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).*
#p < 0.05 statistically significant compared to Veh+AEA

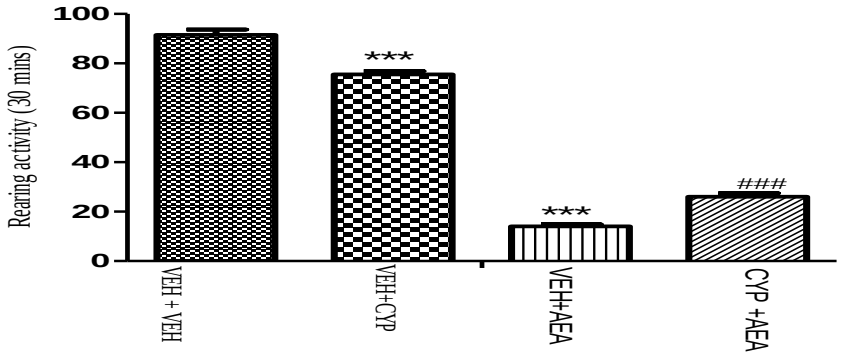

Fig. 14. Effect of cyproheptadine on AEA activity on NIB rearing in mice

Bars represent mean values with a standard error of mean ± SEM.
VEH, CYPRO, and AEA represent vehicle (normal saline, 10 µl/kg, i.p.), diazepam (2 mg/kg, i.p.), cyproheptadine (2 mg/kg, i.p.) and aqueous extract of E.alba, respectively, n=6
****p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test.*
###p < 0.001 statistically significant compared to Veh+AEA

3.4.3 Effect of Cyproheptadine on AEA activity on NIB in mice

The effect of cyproheptadine (2 mg/kg,.i.p.) on the inhibition of locomotion by AEA was investigated in mice. cyproheptadine inhibited locomotion, grooming and rearing behaviour when compared to the vehicle-treated as control group. The results obtained showed that the AEA (400 mg/kg, i.p.) significantly [F (4, 25) = 765, p < 0.0001)] reduced the number of locomotion, grooming and rearing compared to the vehicle-treated control group. The standard drug cyproheptadine (2 mg/kg, i.p.) significantly (p < 0.0001) reduced the number of locomotion, grooming and rearing compared to the vehicle-treated control (Figs 12-14) [32, 33].

4. DISCUSSION AND CONCLUSION

In this study the median acute toxicity (LD_{50}) of the compound AEA was found to be 1,264 mg/kg (i.p.) and 3,807 mg/kg (oral) respectively, showing moderate therapeutic index. Investigation of toxicity is an important step in the toxicological investigation of any substance, it should not be regarded as a biological constant, because some factors could account for variable outcomes such as animals species and strain, age, gender, diet, bedding, ambient temperature and the time of the day [23]. Our results agree with the report of Dreisbach et. al., indicating that LD_{50} is the amount of chemical that will kill approximately 50% of the group of animals [24].

The study showed that the AEA demonstrated considerable inhibitory influence on the central nervous system [22], which demonstrated-dose dependent decrease with a reduction in rearing, grooming and locomotion($p< 0.0001$), suggesting that AEA has either sedative or skeletal muscle relaxation activity or both properties together [25,26].

The antipsychotic effects of AEA (100, 200 and 400 mg/kg) were investigated using 4 different animal models; amphetamine induces hyperlocomotion, swimming-induced grooming, Apomorphine-induced climbing and haloperidol induces catalepsy in mice. Amphetamines are a group of synthetic psychoactive drugs called central nervous system (CNS) stimulants that enhance the release of dopamine and inhibits is reuptake of dopamine [27]. The results of amphetamine induce hyperlocomotion test shows that AEA at test doses significantly ($p< 0.0001$) reduced the frequency of locomotion when compared with the negative control. This signifies the antipsychotic effect, which demonstrates the ability to suppress the abnormal motor behaviour that was exhibited in psychosis. Catalepsy is a motor feature that is seen following the blockage of dopamine receptors (mostly D_2 and D_1) by potent antipsychotic medications which results into a sign of extrapyramidal effects (Skf et. al., 1992; Van Wimersma Greidanus et. al., [28]. In this study, the catalepsy test shows that AEA potentiates the cataleptic effect induced by haloperidol ($p < 0.0001$). In apomorphine induce climbing test shows that AEA inhibit apomorphine-induce climbing behavior compared to the negative control ($p< 0.0001$), which supports the central activity of AEA and might be related to anti-dopaminergic effect and suggestive of AEA contains anti-dopaminergic

compound. Hence the use of the plant (EA) in the treatment of mental illness by traditional healers may be responsible for the observed activity in this study.

This study also demonstrated the mechanism of action of AEA to act via D_2 and serotonergic receptor blockage, using the following antagonist; haloperidol, atropine and cyproheptadine. The study has shown that clinical antipsychotics act as D2 receptor blockers and antipsychotic potency is correlated with their capacity of binding to D2 receptor [29]. Antipsychotics like risperidone and clozapine also block the serotonin (5-HT) system, which helps to reduce the extrapyramidal effects [30]. In results in this study shows that the effects of haloperidol on AEA have a significant ($p < 0.0001$) effect compared to the negative control while the effect of diazepam is also significant ($p < 0.0001$) to the control. The effect of cyproheptadine on AEA shows significant ($p < 0.0001$) antihistaminergic effects compared to the negative control, which has been found useful in the treatment of psychotic features (Goudie, [31]. The findings in this study suggest that the plant possess antipsychotic potential via dopamine and serotonin receptor. The study also provides the scientific basis for the use of the leaves of the plant in the treatment of mental illness in African traditional medicine.

Hence this study shows that AEA is moderately toxic and suggested that AEA exhibits depressive activity in mice. Moreover, this study showed that the AEA exhibited antipsychotic activity in mice. In addition, the mechanism of action was found to be probably via attenuation of dopaminergic and serotonergic activity in the brain. Thus, the CNS effects of the AEA in this research inferentially established the pharmacological basis for the use of the plant ethnomedicinal to treat psychosis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethical clearance for this research was obtained through the Faculty Postgraduate Committee and all animal experiment was carried out in strict compliance with the National Institute of Health (NIH, 1996) as implemented by the OAU Research Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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