

Original Research Article

**PSYCHONEUROPHARMACOLOGICAL PROPERTIES OF ECLIPTA ALBA (LINN)  
IN MICE**

**ABSTRACT**

*Ecliptaalba* L.(EA) has been found useful ethnomedicinally in the treatment of many medical conditions. This study aimed to evaluate the acute toxicity profile, behavioural activity, antipsychotic and mechanism of action of the aqueous extract of *Eclipta Alba* (AEA). Using Lorke's method the LD<sub>50</sub> of AEA was 1,264 mg/kg (*i.p*) and 3807 mg/kg (oral) respectively was obtained, which shows that AEA was moderately toxic. The 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. Furthermore, this study shows that the mechanism of action of AEA (antagonist model: haloperidol, atropine and cyproheptadine) ( $P < 0.0001$ ) may be 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.

**Keyword:** *Ecliptaalba*, Novelty-Induced Behaviours, Acute Toxicity, Aqueous Extract, Antipsychotic, Amphetamine, Catalepsy, Climbing, Mechanism, Haloperidol, Apomorphine,

## 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 (Amos et al., 2001). 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 with ethnomedicinal significance (Azwanida, 2015). Studies have reported that *Eclipta alba* (L.) contains a wide range of active chemicals which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes and triterpenoids. The leaves contain stigmasterol,  $\beta$ -terthienyl-methanol, wedelolactone, dimethylwedelolactone and demethylwedelolactone-7-glucoside (Soni, 2017). 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 (Da, Pooja, 2020). EA has been reported to be useful as a cathartic for jaundice and for skin diseases (Singh Brar & Rupamjot Gill, 2013). The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator (Lans, 2007). It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and is popularly used to enhance memory and learning (Vudara & Vedagiri, 2019). The plant has a reputation as an antiaging agent in Ayurveda (Shukla et al., 2012). Externally it is used for inflammation (Singh et al., 2010), minor cuts, burns and fresh leaf juice is considered very effective in stopping bleeding (Mukherjee et al., 1995). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections (Mukherjee et al., 1995). The water extract of EA (whole plant) exhibited the most potent inhibitory activity against HIV-1 integrase (HIV-1 IN) (Ali et al., 2011; Jahan et al., 2014). Vedic Guard contains EA as a major ingredient in treatments of gastrointestinal disorders (Razdan et al., 2008). It was reported that taking the juice of EA with honey helps to prevent the onset of

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senility, and its oil as the best-medicated massage oil for rejuvenation therapies (Jadhav et al., 2009). EA activities were associated with the central nervous system (Dalal et al., 2010); 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 of the plant using standard models.

## MATERIALS / METHODS

### Plant Collection and Identification

The fresh leaves ~~plants~~ of *Ecliptaalba*L 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.

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### How water extract was obtained?

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### 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.

### 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.

## **General experimental design**

### **Acute toxicity study**

The acute toxicity of AEA was demonstrated in mice using the intraperitoneal (*ip*) 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 30 minutes and then 24 hours for the lethal effects (death). The LD<sub>50</sub> of AEA was estimated as the geometric mean of the lowest dose causing death and the highest dose causing no death following the formula:

$LD_{50} = \sqrt{\text{maximum dose producing 0\% death} \times \text{minimum dose producing 100\% death}}$ . (Lorke's 1983).

### **Experimental Behavioural study:**

#### **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

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appropriate standard drug: Diazepam (DZP) (2 mg/kg, *ip*). All treatments were made by intraperitoneal (*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.

### **Experimental design for antipsychotic**

Animals were randomly selected into 5 groups (n=5). Group I servesserve 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), Apomorphine (APO) and Amphetamine (AMP)). All treatments were made by intraperitoneal (*i.p.*) route.

### **Effect of the AEA on experimental psychosis**

#### **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 tedment 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 120 min 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 (Navarro et al., 1997; Oyemitan et al., 2015)..

#### **Effect of AEA on Apomorphine-induced climbing test.**

The method described by (Davis,(1974); Seong et al.,(2007) 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 postAPO injection (2 mg/kg, i.p.).

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#### **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°C) for about 3 min, the animals were pre-treated following the standard protocol. Afterward, the animals were towel-dried for 20 second 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(Chester and Jackson 1981).

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#### **Amphetamine-induced hyperlocomotiontest**

Locomotor activity was assessed as described by (Salahpour et al.,(2008). 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 crosseswithin 15 min. AMP (2 mg/kg, i.p.) was used as the positive control.

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### 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 antagonistson AEA in NIB (locomotion, rearing, grooming)to explore the possible neurotransmitters or pathways through which the AEAexerts its effects and probable mechanism of action. Fourgroups (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, *ip*), ATR (2 mg/kg, *ip*) and CYP (2 mg/kg, *ip*), group III received the vehicle and 400 mg/kg of AEA, *ip*, andgroup IV received a dose of antagonist and 400 mg/kg of AEA, *ip*. Eachmousewas pre-treated with the antagonist for 15 minutes after which they were observed for NIB for ~~thirty~~ 30minutes (Kulkarni *et al.*, 2013).

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### Statistical analysis

The results were expressed as mean (SEM). All parametric tests were ~~analysed~~ 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.).

## RESULTS

### 1) Acute toxicity:

The results of the acute toxicity study indicate that the LD<sub>50</sub> of the AEA was calculated to be 1,264mg/kg and 3807 mg/kg for the intraperitoneal and oral routes, respectively. Table 1 and 2.

**Table 1: Intraperitoneal route Lethal Dose Toxicity Profile of AEA in mice using Lorke's method (LD<sub>50</sub>)**

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

$$\begin{aligned}LD_{50} (i.p) &= \sqrt{(A \times B)} \\ &= \sqrt{(1600 \times 1000)} \\ &= 1264 \text{ mg/kg.}\end{aligned}$$

**Table 2: Oral route Lethal Dose Toxicity Profile of AEA in mice using Lorke's method (LD<sub>50</sub>)**

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

$$\begin{aligned}
 LD_{50} (i.p) &= \sqrt{(A \times B)} \\
 &= \sqrt{(2,900 \times 5000)}, \\
 &= 3807 \text{ mg/kg.}
 \end{aligned}$$

## 2) Behavioural study

### 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 (Figure 1, 2, 3).

## 3) Antipsychotic effects of AEA

### a. 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.*)

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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.

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#### **b. 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).

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#### **c. 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 (Figure 4).

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#### **d. 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 (2mg/kg, *i.p.*) significantly ( $p < 0.0001$ ) reduced swimming-induced grooming behavior compared to vehicle.

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The result presented in Figure 5.

#### 4) Mechanism of action of AEA using some Antagonists Model of AEA in Mice

##### a. Effect of Haloperidol on ~~AEA activity~~ 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 Figures 6-8.

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##### b. Effect of Atropine on AEA activity on NIB in mice.

The effect of Atropine (2mg/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] 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 (Figures 9-11).

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##### c. Effect of Cyproheptadine on AEA activity on NIB in mice.

The effect of cyproheptadine (2mg/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

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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 (Figures 12-14).

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### 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 3807 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 (Fuhrman & Fuhrman, 2017). 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 (Saganuwan, 2017).

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The study showed that the AEA demonstrated considerable inhibitory influence on the central nervous system (Akanmu et al., 2021), 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 (Ayoka et al., 2006; Dhayabaran & Florance, 2012).

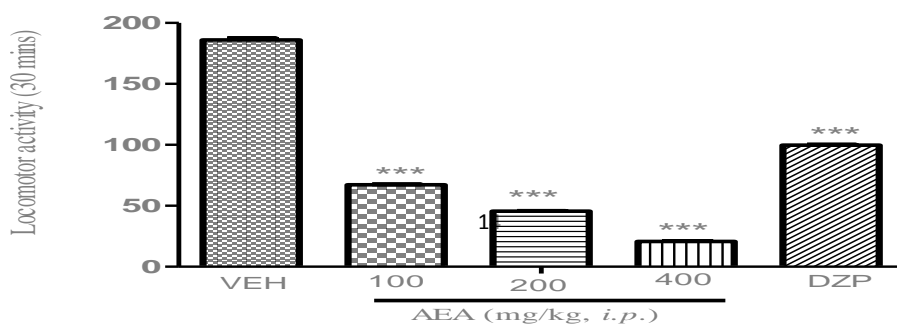
The antipsychotic effects of AEA (100, 200 and 400 mg/kg) were investigated using 4 different animal models; amphetamine induces hyperlocomotion, swimming-induced grooming, Apomorphine-induced climbing and haloperidol induces catalepsy in mice. Amphetamines are a group of synthetic psychoactive drugs called central nervous system (CNS) stimulants that enhance the release of dopamine and inhibit its reuptake of dopamine (J.M Ritter, 2016). 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., 1989). 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-induced 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  $D_2$  receptor blockers and antipsychotic potency is correlated with their capacity of binding to  $D_2$  receptor (Meltzer, 1991). Antipsychotics like risperidone and clozapine also block the serotonin (5-HT) system, which helps to reduce the extrapyramidal effects (Kaur et al., 2010). 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, 2008). 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.

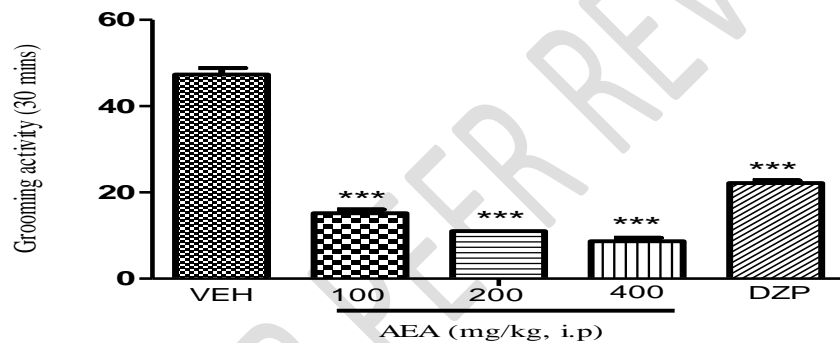


**Figure 1: Effect of AEA on Novelty-induced Locomotion in Mice**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, AEA and DZP represent vehicle (normal saline, 10  $\mu$ l/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).



**Figure 2: Effect of AEA on Novelty-induced Grooming in Mice.**

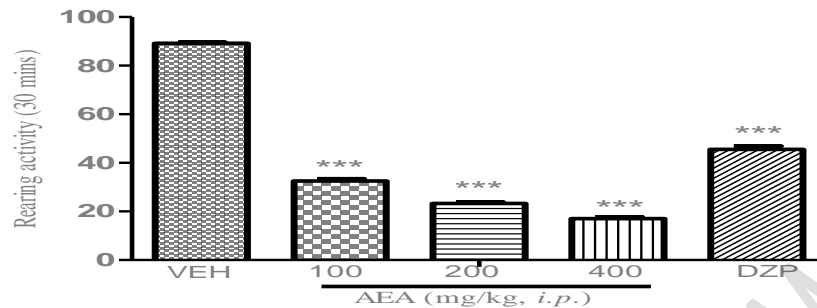
Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP and AEA represent vehicle (normal saline, 10  $\mu$ l/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).

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**Figure 3: Effect of AEA on Novelty-induced rearing in Mice**

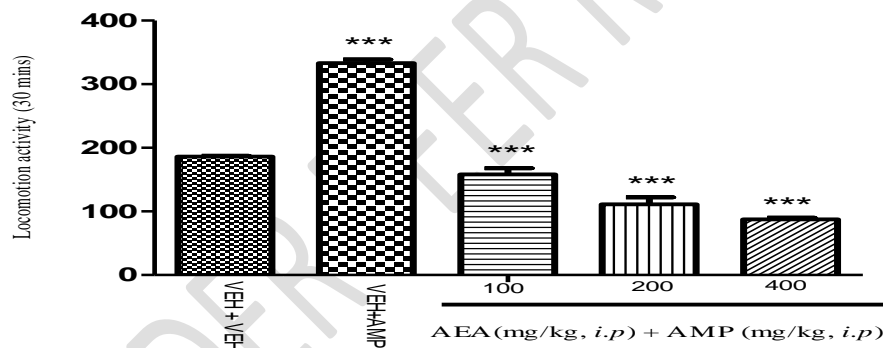
Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), diazepam (2 mg/kg, *i.p.*), and aqueous extract of *E. aAlba*, respectively, n=6.

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

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**Figure 4: Effect of AEA on the amphetamine-induced hyperlocomotion in Mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP, AMP and AEA represent vehicle (normal saline 10  $\mu$ l/kg, *i.p.*), Amphetamine (2mg/kg, *i.p.*) and aqueous extract of *E. aAlba*, respectively, n=6.

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

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**Table3: Effect of AEA on haloperidol-induced catalepsy in Mice**

Treatment	15 Mins	30 Mins	45min	60 Min	90 Min	120 Min
<b>VEH + VEH</b>	0.17 ± 0.11	0.17 + 0.11**	0.08 ± 0.08***	0.17 ± 0.11***	0.25 ± 0.11	0.25 ± 0.11*
<b>VEH + HAL</b>	0.42 ± 0.08	0.67 + 0.17	1.33 ± 0.17**	1.25 ± 0.11***	0.92 ± 0.24	0.67 ± 0.17
<b>AEA 100 + HAL</b>	0.67 ± 0.17	1.75 ± 0.17	3.17 ± 0.2**	3.33 ± 0.17***	2.50 ± 0.26	2.17 ± 0.21***
<b>AEA 200 + HAL</b>	0.67 ± 0.17	1.75 ± 0.17	3.17 ± 0.2*	3.33 ± 0.17***	2.50 ± 0.26	2.17 ± 0.21***
<b>AEA 400 + HAL</b>	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, 10 µl/kg), n=6 and aqueous extract of *E. eliptica* alba, respectively, n=6. Catalepsy score express as mean ± SEM.

\* = p<0.05 compare with vehicle (normal saline)

\*\* = p<0.001 compare with vehicle (normal saline)

\*\*\* = P<0.0001 compare with vehicle (normal saline) using One-way analysis of variance (ANOVA) Student-Newman-Keul's test.

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**Table 4: Effect of Apomorphine- induce climbing on AEA in Mice.**

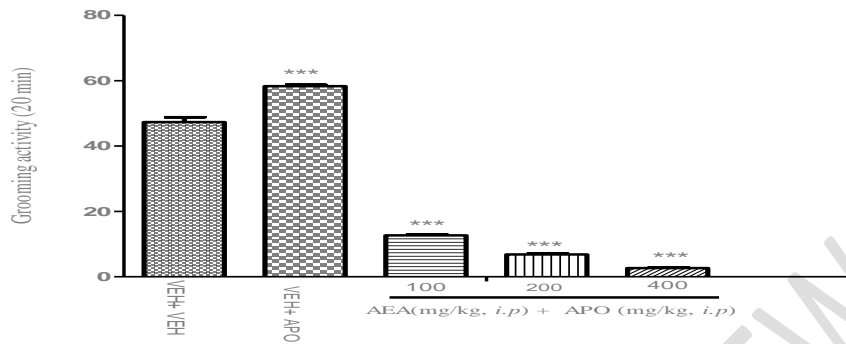
Treatment	10 Min	20 Min	30 Min	40 Min	50 Min	60 Min
VEH	3.7 ± 0.2**	3.8 ± 0.2***	3.8 ± 0.2	3.5 ± 0.3***	3.8 ± 0.2***	3.8 ± 0.2*
VEH + APO	5.5 ± 0.2**	5.7 ± 0.2	6.0 ± 0.0	5.5 ± 0.2	5.5 ± 0.2	4.8 ± 0.2
AEA 100 + APO	2.8 ± 0.2***	2.3 ± 0.2***	2.8 ± 0.3	3.3 ± 0.3***	3.7 ± 0.2***	3.7 ± 0.2*
AEA 200 + APO	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 *EcliptaE.alba*, respectively, n=6. The climbing score expresses as mean ± SEM.

\* = p<0.05 compare with vehicle (normal saline)

\*\* = p<0.001 compare with vehicle (normal saline)

\*\*\* = P<0.0001 compare with vehicle (normal saline) using One-way analysis



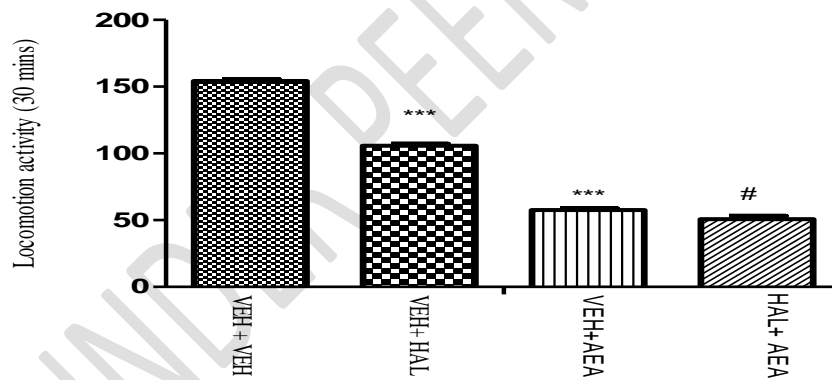
**Figure 5: Effect of Apomorphine Swimming-Induced Grooming Activity on AEA in Mice.**

VEH, APO and AEA represent the vehicle (normal saline, 10  $\mu$ l/kg), Apomorphine (2mg/kg, *i.p.*) and aqueous extract of *E. elipta alba*, respectively, n=6. Score expressed as mean  $\pm$  SEM.

\* = p<0.05 compare with vehicle (normal saline)

\*\* = p<0.001 compare with vehicle (normal saline)

\*\*\* = P<0.0001 compare with vehicle (normal saline) using One-way analysis of variance (ANOVA, Student-Newman-Keul's test).



**Figure 6: Effect of Haloperidol on AEA activity on NIB locomotion in mice.**

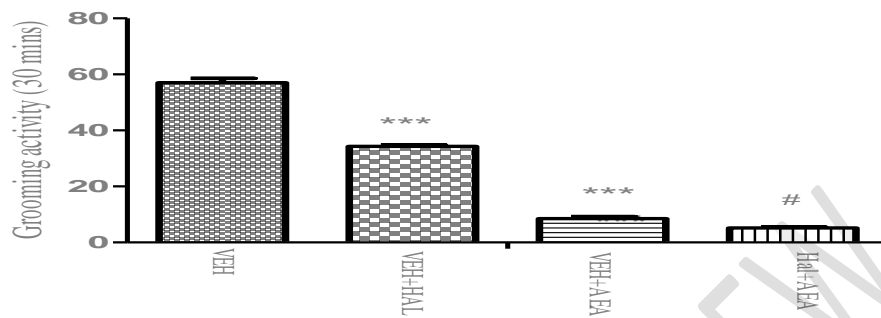
Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP, Hal and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), haloperidol (2mg/kg) and aqueous extract of *E. aAlba*, 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

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**Figure 7: Effect of Haloperidol on AEA activity on NIB grooming in mice.**

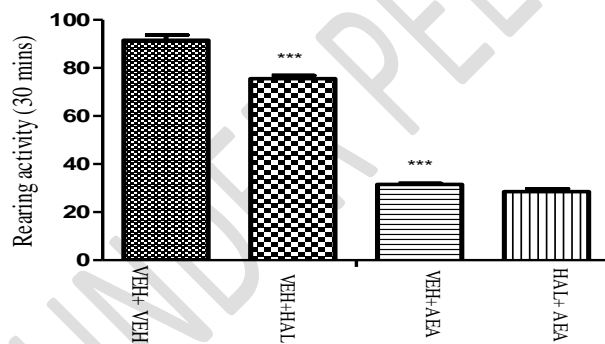
Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP, Hal and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), haloperidol (2mg/kg) and aqueous extract of *E. aAlba*, 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

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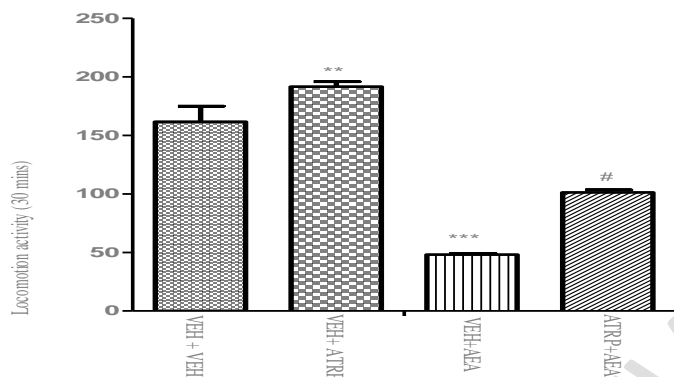
**Figure8: Effect of Haloperidol on AEA activity on NIB rearing in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, DZP, Hal and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), haloperidol (2mg/kg) and aqueous extract of *E. aAlba*, respectively, n=6.

\*\*\*p< 0.01 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).

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**Figure 9: Effect of Atropine on AEA activity on NIB locomotion in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, ATRP and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), Atropine (2mg/kg, *i.p.*) and aqueous extract of *E. aAlba*, respectively, n=6.

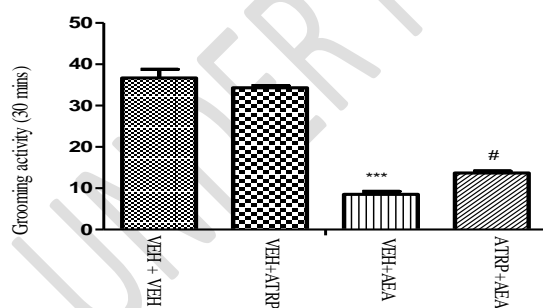
\*\* p < 0. 01 when compared with vehicle (ANOVA, Student-Newman-Keul's test).

\*\*\*p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).

### P < 0.001 statistically significant compared to Veh+AEA

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**Figure 10: Effect of Atropine on AEA activity on NIB grooming in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

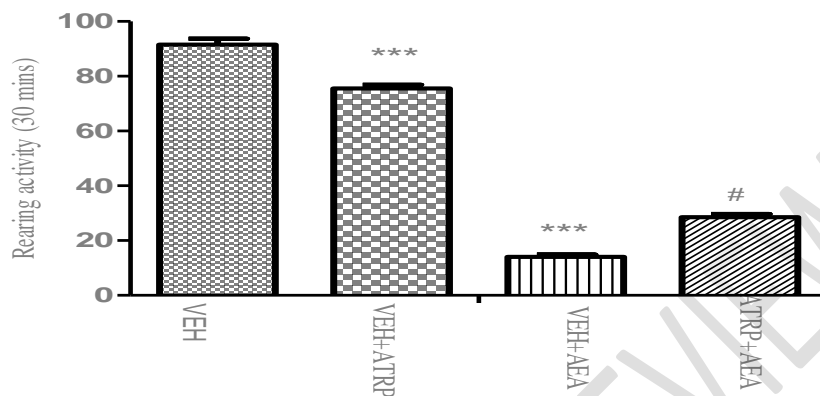
VEH, Atrp and EEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), Atropine (2mg/kg, *i.p.*) and aqueous extract of *E. aAlba*, respectively, n=6.

\*\*\*p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).

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## P<0.01 statistically significant compared to Veh+AEA.



**Figure 11: Effect of Atropine on AEA activity on NIB rearing in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, Atrp and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), Atropine (2mg/kg, *i.p.*) and aqueous extract of *E. aAlba*, respectively, n=6.

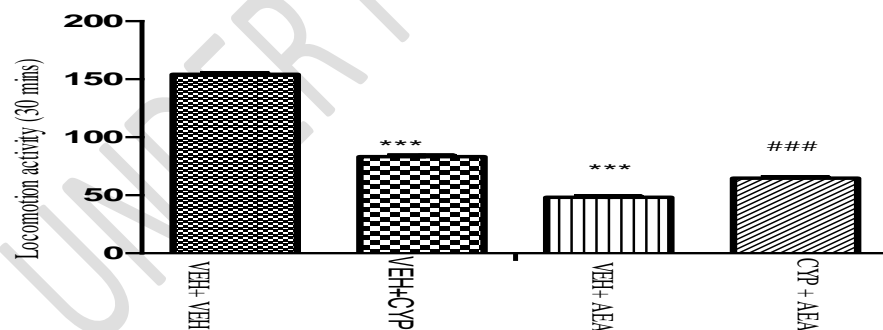
\*\*\*p< 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).

### P<0.001 statistically significant compared to Veh+AEA.

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**Figure 12: Effect of Cyproheptadine on AEA activity on NIB locomotion in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

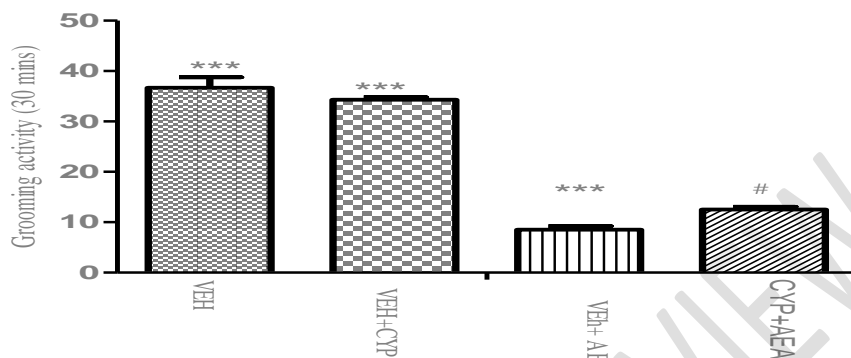
VEH, cypro, and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), Cyproheptadine (2 mg/kg, *i.p.*) and aqueous extract of *E. aAlba*, respectively, n=6.

\*\*\*p< 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test).

### P<0.001 statistically significant compared to Veh+AEA

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**Figure 13: Effect of Cyproheptadine on AEA activity on NIB grooming in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, cypro, and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), cyproheptadine (2mg/kg, *i.p.*) and aqueous extract of *E. alba*, respectively, n=6.

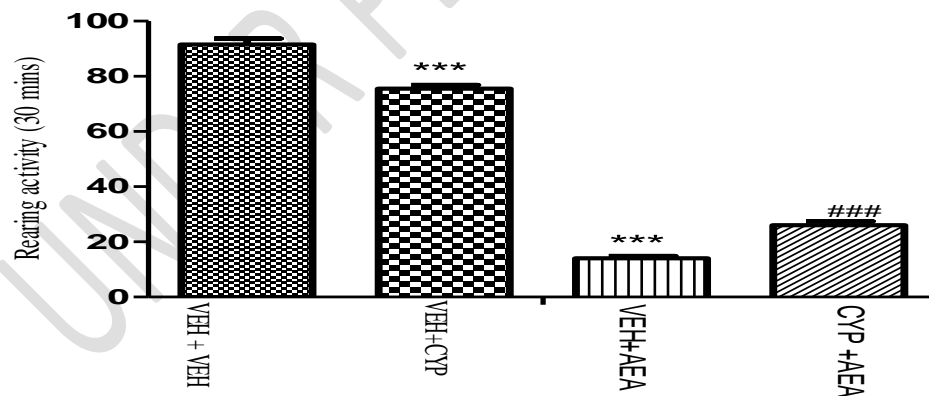
\*\*\*p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test). #p < 0.05 statistically significant compared to Veh+AEA.

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**Figure 14: Effect of Cyproheptadine on AEA activity on NIB rearing in mice.**

Bars represent mean values with a standard error of mean  $\pm$  SEM.

VEH, cypro, and AEA represent vehicle (normal saline, 10  $\mu$ l/kg, *i.p.*), diazepam (2 mg/kg, *i.p.*), cyproheptadine (2mg/kg, *i.p.*) and aqueous extract of *E. Alba*, respectively, n=6.

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\*\*\*p < 0.001 statistically significant compared to vehicle (ANOVA, Student-Newman-Keul's test. ###P < 0.001 statistically significant compared to Veh+AEA.

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## References:

Akanmu, A. O., Sodipo, O. A., Sandabe, U. K., Shamaki, B. U., Balogun, S. T., & Jubrin, J. (2021).

Novelty-induced behavior and memory enhancing activities of aqueous and ethanol extracts of *Solanum incanum* Linn. fruits in mice. *African Journal of Pharmacy and Pharmacology*, 15(2), 33–42. <https://doi.org/10.5897/ajpp2020.5210>

Ali, H., Khyber, M. T., & Khyber, M. S. (2011). Antimicrobial potentials of *Eclipta alba* by disc diffusion method. *African Journal of Biotechnology*, 10(39), 7658–7667. <https://doi.org/10.5897/AJB11.454>

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Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O., Akanmu, M. A., & Ukponmwan, O. E. (2006).

Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiaceae) in mice and rats. *Journal of Ethnopharmacology*, 103(2), 166–175. <https://doi.org/10.1016/j.jep.2005.07.019>

Azwanida. (2015). *Medicinal & Aromatic Plants A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation*. 4(3), 3–8. <https://doi.org/10.4172/2167-0412.1000196>

Berridge, K. C., & Aldridge, J. W. (2000). Super-stereotypy I: Enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Journal of Neuroscience*, 37(3), 194–204. [https://doi.org/10.1002/1098-2396\(20000901\)37:3<194::AID-SYN3>3.0.CO;2-A](https://doi.org/10.1002/1098-2396(20000901)37:3<194::AID-SYN3>3.0.CO;2-A)

Chartoff, E. H., Marck, B. T., Matsumoto, A. M., Dorsa, D. M., & Palmiter, R. D. (2001). Induction of stereotypy in dopamine-deficient mice requires striatal D1 receptor activation. *Proceedings of the National Academy of Sciences of the United States of America*, 98(18), 10451–10456. <https://doi.org/10.1073/pnas.181356498>

Chinedu, E., Arome, D., & Ameh, F. S. (2013). A new method for determining acute toxicity in animal

models. *Toxicology International*, 20(3), 224–226. <https://doi.org/10.4103/0971-6580.121674>

Da, Pooja, R. S. G. (2020). Pharmacological and therapeutic importance of *Eclipta alba* (Bili garuga): A review Pooja DA, Raviraja Shetty G and Rajani MB. *Journal of Pharmacognosy and Phytochemistry*, 9(4), 577–579. [www.phytojournal.com](http://www.phytojournal.com)

Dalal, S., Kataria, S. K., Sastry, K. V., & Rana, S. V. S. (2010). Phytochemical Screening of Methanolic Extract and Antibacterial Activity of Active Principles of Hepatoprotective Herb, *Eclipta alba*. *Ethnobotanical Leaflets*, 14(10), 248–258.

Davis, J. M. (1974). Dose equivalence of the anti-psychotic drugs. *Journal of Psychiatric Research*, 11(2), 65–69. [https://doi.org/10.1016/0022-3956\(74\)90071-5](https://doi.org/10.1016/0022-3956(74)90071-5)

Dhayabaran, D., & Florance, J. (2012). Anticonvulsant activity of alcoholic root extract of *Cardiospermum halicacabum*. *Brazilian Journal of Pharmacognosy*, 22(3), 623–629.

Fuhrman, G. J., & Fuhrman, F. A. (2017). EFFECTS OF TEMPERATURE ON THE ACTION OF DRUGS1,2 Further Annual Reviews. *Bulgarian Journal of Veterinary Medicine*, 20(4), 291–318. [www.annualreviews.org](http://www.annualreviews.org)

Gobira, P. H., Ropke, J., Aguiar, D. C., Crippa, A. S., & Moreira, A. (2013). Animal models for predicting the efficacy and side effects of antipsychotic drugs. *Revista Brasileira de Psiquiatria*, 35(3), 132–139. <https://doi.org/10.1590/1516-4446-2013-1164>

Goudie, A. J. (2008). Cyproheptadine resembles clozapine in vivo following both acute and chronic administration in rats (*Journal of Psychopharmacology* 21, 2, DOI: 10.1177/0269881107067076). *Journal of Psychopharmacology*, 22(6), 698. <https://doi.org/10.1177/0269881108096191>

J.M Ritter, R. F. (2016). *RANG AND DALE'S Pharmacology*.

Jadhav, V. M., Thorat, R. M., Kadam, V. J., & Sathe, N. S. (2009). *Eclipta alba* Linn - “Kesharaja”: A Review. *Journal of Pharmacy Research*, 2(8), 1236–1241.

<http://search.ebscohost.com/login.aspx?direct=true&db=aph&AN=43935484&site=ehost-live>

Jahan, R., Al-Nahain, A., Majumder, S., & Rahmatullah, M. (2014). Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae). *International Scholarly Research Notices*, 20(3), 1–22.

<https://doi.org/10.1155/2014/385969>

Kaur, H., Kumar, S., Vishwakarma, P., Sharma, M., Saxena, K. K., & Kumar, A. (2010). Synthesis and antipsychotic and anticonvulsant activity of some new substituted oxa/thiadiazolylazetidinyll/thiazolidinonylcarbazoles. *European Journal of Medicinal Chemistry*, 45(7), 2777–2783. <https://doi.org/10.1016/j.ejmech.2010.02.060>

Lans, C. (2007). Comparison of plants used for skin and stomach problems in Trinidad and Tobago with Asian ethnomedicine. *Journal of Ethnobiology and Ethnomedicine*, 3(3), 1–12.

<https://doi.org/10.1186/1746-4269-3-3>

Meltzer, H. Y. (1991). The mechanism of action of novel antipsychotic drugs. *Schizophrenia Bulletin*, 17(2), 263–287. <https://doi.org/10.1093/schbul/17.2.263>

Moreno, J. L., & González-Maeso, J. (2013). Preclinical models of antipsychotic drug action.

*International Journal of Neuropsychopharmacology*, 16(10), 2131–2144.

<https://doi.org/10.1017/S1461145713000606>

Mukherjee, P. K., Bhakta, T., Pal, S., Pal, M., Saha, B. P., & Das, A. K. (1995). Anti-cholesterolaemic activity of a fraction of *azadirachta indica* leaf extract on rats. *Ancient Science of Life*, 14(3), 150–153.

<http://www.ncbi.nlm.nih.gov/pubmed/22556692>  
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3331234>

Navarro, J. F., Manzanque, J. M., Martín-López, M., & Vera, F. (1997). Daily versus intermittent haloperidol administration: Effects on catalepsy of mice. *Psicothema*, 9(1), 83–87.

- Oyemitan, I. A., Olayera, O. A., Alabi, A., Abass, L. A., Elusiyani, C. A., Oyedeji, A. O., & Akanmu, M. A. (2015). Psychoneuropharmacological activities and chemical composition of essential oil of fresh fruits of *Piper guineense* (Piperaceae) in mice. *Journal of Ethnopharmacology*, *166*(1), 240–249. <https://doi.org/10.1016/j.jep.2015.03.004>
- Razdan, R., Imranulla, & Amar Dev, M. J. (2008). Preventive and curative effects of Vedic Guard against anti-tubercular drugs induced hepatic damage in rats. *Journal of Pharmacognosy*, *4*(15), 182–188.
- Saganuwan, S. A. (2017). Toxicity studies of drugs and chemicals in animals: An overview. *Bulgarian Journal of Veterinary Medicine*, *20*(4), 291–318. <https://doi.org/10.15547/bjvm.983>
- Saganuwan, S. A., Ahur, V. M., & Yohanna, C. A. (2008). Acute toxicity studies of potassium permanganate in Swiss albino mice. *Nigerian Journal of Physiological Sciences : Official Publication of the Physiological Society of Nigeria*, *23*(1–2), 31–35. <https://doi.org/10.4314/njps.v23i1-2.54917>
- Salahpour, A., Ramsey, A. J., Medvedev, I. O., Kile, B., Sotnikova, T. D., Holmstrand, E., Ghisi, V., Nicholls, P. J., Wong, L., Murphy, K., Sesack, S. R., Wightman, R. M., Gainetdinov, R. R., & Caron, M. G. (2008). Increased amphetamine-induced hyperactivity and reward in mice overexpressing the dopamine transporter. *105*(11), 2–7.
- Seong, H. J., Jin, Y. K., & In, W. C. (2007). Effects of newer antipsychotic drugs on apomorphine-induced climbing behavior in mice. *Clinical Psychopharmacology and Neuroscience*, *5*(1), 19–24.
- Shukla, S. D., Bhatnagar, M., & Khurana, S. (2012). Critical evaluation of ayurvedic plants for stimulating intrinsic antioxidant response. *Frontiers in Neuroscience*, *6*(4), 1–12. <https://doi.org/10.3389/fnins.2012.00112>
- Singh, A., Duggal, S., Singh, J., & Katekhaye, S. (2010). *Eclipta alba* Linn. ancient remedy with therapeutic potential. *International Journal of Phytopharmacology*, *1*(2), 57–63. [file:///C:/Users/user\\_2/Downloads/77\\_57-63.pdf](file:///C:/Users/user_2/Downloads/77_57-63.pdf)

- singh Brar & Rupamjot Gill. (2013). Pharmacological Activity and Chemical Constituents of Eclipta Alba. *Global Journal of Medical Research Pharma, Drug Discovery, Toxicology and Medicine*, 13(7), 1–7.
- Skf, D.-, Siobhan, A., & Waddington, J. L. (1992). D-1 dopamine receptors and the topography of unconditioned motor behaviour: studies with the selective, 'full efficacy' benzazepine D-1 agonist SKF 83189. *Journal of Psychopharmacology*, 6(1), 50–60.
- Soni, S. (2017). Eclipta alba ( L .) An Ethnomedicinal Herb Plant , Traditionally Use in Ayurveda. *Journal of Horticulture*, 4(3), 3–4. <https://doi.org/10.4172/2376-0354.1000208>
- Tchernichovski, O., Benjamini, Y., & Golani, I. (1996). Constraints and the emergence of “free” exploratory behavior in rat ontogeny. *Journal of Behaviour*, 33(7), 519–539. <https://doi.org/10.1163/156853996X00198>
- Van Wimersma Greidanus, T. B., Maigret, C., Torn, M., Ronner, E., Van der Kracht, S., Van der Wee, N. J. A., & Versteeg, D. H. G. (1989). Dopamine D-1 and D-2 receptor agonists and antagonists and neuropeptide-induced excessive grooming. *European Journal of Pharmacology*, 173(2–3), 227–231. [https://doi.org/10.1016/0014-2999\(89\)90527-X](https://doi.org/10.1016/0014-2999(89)90527-X)
- Vasse, M., Chagraoui, A., & Protais, P. (1988). Climbing and stereotyped behaviours in mice require the stimulation of D-1 dopamine receptors. *European Journal of Pharmacology*, 148(2), 221–229. [https://doi.org/10.1016/0014-2999\(88\)90567-5](https://doi.org/10.1016/0014-2999(88)90567-5)
- Vudara, R., & Vedagiri, K. (2019). Pharmacological Study of the Methanolic Whole Plant Extract of Eclipta alba Against Ischemic Reperfusion Injury on Kidney of Sprague Dawley Rats. *International Journal of Pharmacognosy and Phytochemical Research*, 11(3), 105–115. <https://doi.org/10.25258/phyto.11.3.3>

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