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Evaluation of the psychopharmacological properties and neural mechanisms of action of the ethanol extract of leaves of *Triumfetta cordifolia* in mice

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Abstract

This study was carried out to investigate neurobehavioral properties and the underlying neural mechanisms of action of the ethanol extract of leaves of *Triumfetta cordifolia* (EETC) on behavioral models in mice. The acute toxicity test of EETC was assessed using Locke's method. Thereafter, neurobehavioral property of EETC (4.4, 8.8 and 17.5 mg/kg) administered intraperitoneally (i.p.) was evaluated on novelty-induced rearing, grooming and locomotor using open-field test; cognitive enhancing effect was evaluated using Y-maze test. The anxiolytic and sedative effects were assessed using elevated-plus maze and hole board tests respectively. Moreover, the potential underlying neural mechanisms of EETC was carried out using neurotransmitter receptor antagonists: haloperidol (0.2 mg/kg), yohimbine (1 mg/kg), propranolol (0.2 mg/kg), cyproheptadine (0.5 mg/kg) and atropine (0.5 mg/kg) on novelty-induced rearing, grooming, locomotor and hole board tests. Acute toxicity test carried out revealed the LD_{50} of the extract was estimated to be 282 mg/kg, i.p. EETC significantly ($p < 0.05$) reduced rearing, grooming and locomotor activity in the open-field test. Moreover, EETC reduced head dipping activity in the hole board test, suggesting sedation. EETC did not exhibit anxiolytic and memory enhancing effects in comparison to controls. Pretreatments with haloperidol, yohimbine, propranolol, cyproheptadine and atropine significantly potentiated the inhibitory effects of EETC on rearing and grooming, but reversed its effect on locomotion in the open-field test respectively. In conclusion, the findings suggest that EETC possesses central nervous system depressant activity and the effect might be related to modulation of dopaminergic, noradrenergic, serotoninergic and cholinergic neurotransmissions.

Keywords: *Triumfetta cordifolia;* Toxicity; Rearing; Grooming.

INTRODUCTION

Triumfetta cordifolia A. Rich is an erect perennial shrub belonging to the *Tiliaceae* family [1]. It is distributed in different parts of the world including Bermuda, South America, Florida and West African [2]. It grows on moister areas of Tropical Africa locally on wooded grassland, secondary forest, edges and clearings of wet forest, riverine forest, marshy locations and roadsides [1,2].

The sap of leafy twigs of *Triumfetta cordifolia* is widely used in ethnomedicine for the treatment of digestive disorders, diarrhoeal, dysentery, ulcerogenic conditions, diabetes, asthenia, marasmus, rhinitis, hepatitis, lumbago, muscle pain, backache, fever, inflammation and mental disorders

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[1,2]. The decoction of the flowers is used for malarial [2]. The root is used for the treatment of venereal diseases, liver and kidney disorders; while the fruit can also be macerated in water or local alcohol for the treatment delayed labour [1]. Most of the biological effects ascribed to *Triumfetta cordifolia* extracts have been attributed to its primary bioactive constituents, derived from its leaves, fruits and roots [3]. The chemical constituents isolated from the leaves, flowers and roots of *T. cordifolia* including maslinic acid, betulinic acid, alkaloids, tannins, saponins, steroids, terpenes, stigmasterol, tormentic acid, oleanolic acid, cardiac glycosides and flavonoids like quercetin have been reported to demonstrate diverse biological activities [3, 4]. Accordingly previous studies have shown that *T. cordifolia* possess antidiarrhoeal, antiulcerogenic, antidiabetic, antibacterial, antimalarial, antifungal and anti-inflammatory properties [3, 5, 6]. Aqueous extract of the stem has also been shown to induce weight loss and protect against hyperlipidemia in guinea pigs [7]. In addition, maslinic acid and its oxidized derivative, betulin (betulinic acid) are known to have anti-HIV activity [8]. Preclinical studies have also reported that *T. cordifolia* demonstrated strong antioxidant activity, as it was shown to be efficient in scavenging free radicals [4]. Thus, justifying the role of animal self-medicative behavior as source of possible epigenome modulators and may aid in the treatment of neurobehavioral deficits [9]. Hence, this study was carried out to evaluate the neurobehavioral effects and neural mechanisms of action of the ethanol extract of leaves of *T. cordifolia* (EETC) in mice.

EXPERIMENTAL

Plant material. The leaves of *Triumfetta cordifolia* was collected and taxonomically identified by Mr O.A. Ugbogu and Mr O.S. Shasanya at the Forestry Reserve Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria with an FHI No.109530.

Preparation of extract. Air-dried leaves (300 g) were pulverized and soaked in 750 mL of 50% ethanol for 48 h. The filtrate was concentrated with a rotary evaporator to semi-solid residue at 38°C and evaporated to dryness to produce a solid residue, which was kept in the desiccator. However, the yield of the extract was 10.8 g with reference to the powdered leaves. The dried extract was subsequently dispensed in distilled water at different concentrations for various experiments.

Drug and chemicals. Diazepam (Hoffman-La Roche, Switzerland); atropine, cyproheptadine (Shalina Lab, India); propranolol, haloperidol and yohimbine (Sigma Chemicals Co. St. Louis, Missouri, USA) were used in the study.

Drug preparation. The ethanol extract of leaves of *Triumfetta cordifolia* was dissolved in 5% dimethyl sulfoxide (DMSO). Diazepam, atropine, yohimbine and cyproheptadine were also dissolved in normal saline. Report has shown that 5% concentration of DMSO produced no behavioral affect in rodents [10]. All drugs including vehicle (5% DMSO, 10 mL/kg) were administered intraperitoneally (i.p.).

Experimental animals. Male Swiss mice (20-25 g; 6 weeks old) of either sex were obtained from the Central Animal House, University of Ibadan. The animals were housed five per plastic cage (42 x 30 x 27 cm) at a room temperature (25 ± 1°C) and relative humidity of 60 ± 5% with a 12-h light/dark cycle. They were fed with standard rodent pellet food and water *ad libitum* throughout the experimental period. They were acclimatized for at least 1 week prior to commencement of the experiments. The experimental procedures were performed in accordance with the National institutes of Health (NIH) Guideline for the Care and Use

of Laboratory Animals (Publication No. 85-23, revised 1985).

Acute toxicity tests. Acute toxicity study, which consists of LD50 determination, was carried out according to the method described by Lorke [11]. Briefly, Swiss mice (20-25g) of either sex were used. This method involves an initial dosing of 10, 100 and 1000 mg/kg of extract administered i.p to three groups of animals (n=3) respectively. Thereafter, mortality and general behavior of the treated animals were monitored for 24 h. From the results of the above step, four different doses (200, 400, 600 and 800 mg/kg) were chosen and administered i.p to four groups (n=1) respectively; after which the animals were monitored for 24 h. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death

Behavioral studies

Evaluation of novelty-induced rearing and grooming behaviour of EETC. The effect of the ethanol extract of leave of *T. cordifolia* on rearing and grooming was assessed using the open-field apparatus. Animals were randomly divided into 5 groups (n=6). Mice in group 1, which served as control received vehicle (5% DMSO) (10 mL/kg, i.p.), groups 2-4 were treated with different dose of EETC (4.4, 8.8 and 17.5 mg/kg, i.p.) while group 5 received diazepam (DZP) (2 mg/kg, i.p.). Thirty minutes after, novelty-induced rearing and grooming behavior was assessed individually after single intraperitoneal injection of drug or vehicle, according to the method previously described by [12]. The animals were placed into an opaque Plexiglas observation chamber with one transparent side for observation. Each animal was used only once, with the observation chamber cage cleaned with 70% ethanol and allowed to dry after each assessment to remove olfactory cue from previous animal. The frequency of rearing episodes was recorded using a manual counter and a stopwatch for a period of 30 min. The total frequency was summed up for each animal for the 30 min period of observation. Novelty-induced rearing (NIR) was taken as the number of times the mouse stands on its hind limbs or with its forelimbs against the wall of the observation cage or in the free air, while Novelty-induced grooming (NIG) represent the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions.

Effect of EETC on locomotor activity. The effect of EETC on locomotor activity was assessed using the open-field test (OFT). Animals were randomly grouped into 5 treatment groups (n=6). Mice in group 1 served as normal control and received vehicle (10 mL/kg, i.p.), groups 2-4 received EETC (4.4, 8.8 and 17.5 mg/kg, i.p.) and group 5 received DZP (2 mg/kg, i.p.). The open-field apparatus which consisted of a wooden box (28 x 28 x 25 cm) with visible lines drawn to divide the floor into 16 (7 x 7 cm) equal squares with a frontal glass wall, and placed in a sound free room. The animals were placed in the rear left square and left to explore it. The number of squares crossed with all paws (crossing) was recorded and counted for a period of 5 min as previously described. The observation cage was cleaned with 70% ethanol after each assessment to remove olfactory cue from previous animal [13].

Assessment of hole board exploratory behaviour of EETC in mice. The effect of EETC on the frequency of head dipping behavior was evaluated on the hole-board apparatus according to Vogel *et al*., [14]. The hole board apparatus measures the anxiolytic/sedative exploratory behavioral effects of test drugs, and consists of wooden slabs, containing 16 inspection holes measuring 3 cm diameter with 5 cm between each holes and 50 cm above the ground. Mice were randomly distributed into 5 treatment groups (n=6). Group 1, received vehicle (5%

DMSO, 10 mL/kg, i.p.) and served as normal control, mice in groups 2-4 were treated with EETC (4.4, 8.8 and 17.5 mg/kg, i.p.) and group 5 received DZP (2 mg/kg, i.p.). Thirty minutes after the single intraperitoneal injection of drug or vehicle, the animals were placed individually on top of the wooden slab hole board and the number of head dips/poking by each animal was recorded for a period of 5 min

Evaluation of the effect of EETC on memory performance. The Y-maze test (YMT) was used to assess the effect of EETC on memory performance in mice, based on percentage correct alternations of mice and served as an index of spatial working memory. The apparatus consists of three identical arms (33 × 11 × 12 cm each) in which the arms are symmetrically separated at 120°. Group 1, received vehicle (5% DMSO, 10 mL/kg, i.p.) and served as normal control, mice in groups 2-4 were treated with EETC (4.4, 8.8 and 17.5 mg/kg, i.p.) and group 5 received DZP (2 mg/kg, i.p.) (n=6). Mice were placed in the Y-maze apparatus at the end of arm A and allowed to explore all the three arms (labeled A, B, C) freely for 5 min, taking the following parameters such as the number of arm visits and sequence (alternation) of arm visits visually. Thereafter, the percentage of alternations was calculated as total of alternations / (total arm entries − 2) [15]. After each test session, the observation chamber was cleaned with 70% ethanol to remove residual odor.

Assessment of the Anxiolytic effect of EETC using Elevated plus maze test. Elevated plus maze (EPM) test was used to assess for possible anxiolytic effect of EETC [16]. The apparatus consists of a central square platform (5 x 5 cm) from which emanated two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) directly opposite each other, respectively. The entire apparatus is elevated to a height of 50 cm above floor level. Mice were grouped into 5 treatment groups (n=6). Group 1, received vehicle (5% DMSO, 10 mL/kg, i.p.) and served as normal control, groups 2-4 were treated with EETC (4.4, 8.8 and 17.5 mg/kg, i.p.) while mice in group 5 received DZP (2 mg/kg, i.p.). Thirty minutes after the single intraperitoneal injection of drug or vehicle, mouse was placed at the edge of an open arm, with its head facing the center and allowed to explore the maze for 5 min. During the test period, the following measurements were recorded: the total number of arm entries and the time spent in open and closed arms. An entry with all feet put into one arm is defined as an arm entry in this experiment. Thereafter, the results were expressed as time spent in arms and percentage of number of entries in arms (mean ratio of entries in an arm to total entries in both open and closed arms). The Index of open arms avoidance [IOAA] was determined using IOAA = 100 - (% time spent in open arms + % entries into open arms)/2 as described by [16]. Ethanol (70%) was used to clean the maze after each test session to prevent residual odor bias.

Assessments of the neural mechanisms of actions involved in the neurobehavioral effects of EETC using drug interaction studies in mice. The neural mechanisms involved in the neurobehavioral (rearing, grooming, head dipping and locomotor activities) effects of EETC (17.5 mg/kg, i.p.) was assessed using different neurotransmitter receptor blocker(s) [atropine (muscarinic blocker 0.5 mg/kg), propranolol (β adrenergic antagonist 0.2 mg/kg), yohimbine (α_2 adrenergic antagonist, 1 mg/kg), haloperidol (dopamine D_1 receptor antagonist, 0.2 mg/kg), cyproheptadine (5-HT antagonist, 0.5 mg/kg) as previously described by [17]. Briefly, mice were divided into four (4) treatment groups. Group 1, which served as normal control received vehicle (5% DMSO, 10 mL/kg, i.p.), group 2 was treated with blocker, group 3 received EETC (17.5 mg/kg, i.p.), while group 4 was pre-treated with blocker(s) 15 min before

treatment with EETC (17.5 mg/kg, i.p) respectively. Thereafter (30 min), animals were evaluated for interaction effect (antagonism) on rearing, grooming, head dipping and locomotor behaviors.

Statistical analysis. All data are presented as Mean ± SEM. The results were analyzed by using One-way Analysis of Variance (ANOVA) and Post-hoc test (Newman-Keuls) were carried out to determine the source of significant main effect using GraphPad InStat® Biostatistics software (Graphpad Software, Inc., La Jolla, USA version 4.0). The level of significance for all tests were set at $p \leq 0.05$

RESULTS

Toxicity tests. The LD_{50} of ethanol extract of leaves of *Triumfetta cordifolia* in mice was found to be 282 mg/kg i.p, and this determination was carried out in a 24 h continuous observation.

Effect of ethanol extract of leaves of *Triumfetta cordifolia* on rearing behaviour in mice. The effect of EETC on NIR is shown in Fig 1. Intraperitoneal administration of EETC (4.4 mg/kg, 8.8 mg/kg, and 17.5 mg/kg) significantly ($p < 0.05$) induced a dose dependent decrease in NIR in mice when compared with the vehicle (5% DMSO, 10 mL/kg, i.p) [F (4, 20) = 978.2, P < 0.0001]. Similarly, treatment with DZP (2 mg/kg, i.p.) significantly ($p < 0.05$) reduced NIR relative to vehicle group (Fig 1).

Effect of ethanol extract of leaves of *Triumfetta cordifolia* on grooming behaviour in mice. The effect of EETC on NIG is shown in Fig 2. Treatment with EETC (4.4 mg/kg, 8.8 mg/kg, and 17.5 mg/kg, i.p.) significantly ($p < 0.05$) decreased NIG [F (4, 20) = 38.47, P< 0.0001] when compared with the vehicle control. Also, intraperitoneal treatment with DZP (2 mg/kg, i.p.) significantly ($p < 0.05$) decrease NIG relative to vehicle group (Fig 2).

Effect of ethanol extract of leaves of *Triumfetta cordifolia* on head dipping activity in mice. The effect of EETC on head dipping activity in the hole board test is shown in Fig 3. Pretreatment with EETC (4.4 mg/kg, 8.8 mg/kg, and 17.5 mg/kg, i.p.) significantly ($p < 0.05$) produced a dose-dependent decrease in head dipping behavior similar to DPZ (2 mg/kg, i.p.) in comparison with vehicle control [F (4, 20) = 24.14, P < 0.0001] (Fig 3).

Effect of ethanol extract of leaves of *Triumfetta cordifolia* on locomotor activity in mice. The effect of EETC on locomotor activity based on the number of line crossing in the open-field test in mice is shown in Fig 4. Intraperitoneal administration of EETC (4.4 mg/kg, 8.8 mg/kg, and 17.5 mg/kg) significantly ($p < 0.05$) [F (4, 20) = 71.08, P < 0.0001] reduced the number of line crossing in a dose dependent manner in the OFT relative to vehicle control, suggesting decreased locomotor activity. Moreover, treatment with DZP (2 mg/kg, i.p.) also significantly ($p < 0.05$) reduced locomotor activity when compared with vehicle group (Fig 4).

Effect of the ethanol extract of leaves of *Triumfetta cordifolia* on memory performance in mice. The effect of EETC on memory performance is shown in Fig 5. Intraperitoneal pretreatment with EETC (4.4 mg/kg, 8.8 mg/kg, 17.5 mg/kg) did not produce any significant ($p > 0.05$) effect on memory performance in mice when compared with vehicle control group. However, inraperitoneal injection of DZP (2 mg/kg, i.p) significantly ($p < 0.05$) decreased memory performance, as shown by decreased percentage alternation in the YMT when compared with vehicle group (Fig 5).

Effect of the ethanol extract of leaves of Triumfetta cordifolia on anxiety-like behaviour in mice. The effect of EETC on anxiety-like in the elevated-plus maze test in

mice is shown in Table 1. Intraperitoneal pretreatment with EETC (4.4 mg/kg, 8.8 mg/kg, 17.5 mg/kg) showed no significant ($p > 0.05$) effect on anxiety-like behavior in mice when compared with vehicle control group. However, inraperitoneal injection of DZP (2 mg/kg, i.p) significantly ($p < 0.05$) decreased anxiety-like behavior, as shown by increase in the frequency [$F(4, 20) = 26.96$, $P < 0.0001$] and duration of time spent [$F(4, 20) = 266.1$, $P < 0.0001$] in the open arm as well as decreased in the index of open arm avoidance in the EPM when compared with vehicle group (Table 1).

Effect of atropine on the activity of ethanol extract of leaves of *Triumfetta cordifolia* in mice. The effect of atropine on the neurobehavioral activity of EETC (17.5 mg/kg, i.p) is shown Tables 2 and 3. Intraperitoneal administration of Atropine (0.5 mg/kg) alone demonstrated a significant ($p < 0.05$) decrease in NIR, NIG and head dipping but not locomotor behavior when compared with vehicle controls (Table 2). However, pretreatment with atropine (0.5 mg/kg, i.p.) significantly ($p < 0.05$) reversed the effect of EETC (17.5 mg/kg, i.p) on locomotor activity and potentiated grooming behavior when compared with EETC-treated mice, although no effect was observed on EETC-induced rearing and head dipping (Table 3).

Effect of yohimbine on the activity of ethanol extract of leaves of *Triumfetta cordifolia* in mice. The effect of yohimbine on the neurobehavioral activity of EETC (17.5 mg/kg, i.p) is shown Tables 2 and 3. Intraperitoneal administration of yohimbine (1 mg/kg) alone significantly ($p < 0.05$) reduced NIR, NIG, head dipping and locomotor activities relative to vehicle-treated mice (Table 2). However, pretreatment with yohimbine (1 mg/kg, i.p.) partially reversed the effect of EETC (17.5 mg/kg, i.p) on locomotor activity and potentiated grooming behavior when compared with EETC-treated mice (Table 3). No significant effect was observed on EETC-induced rearing and head dipping (Table 3).

Effect of cyproheptadine on the activity of ethanol extract of leaves of *Triumfetta cordifolia* in mice. Intraperitoneal treatment with cyproheptadine (0.5 mg/kg) alone produced a marked decrement in NIR, NIG, head dipping and locomotor activities in comparison with vehicle control groups Table 2. Pretreatment with cyproheptadine (0.5 mg/kg, i.p.) significantly ($p < 0.05$) potentiated the effects of EETC (17.5 mg/kg, i.p) on NIR and NIG, but reversed the effect of EETC on locomotor activity when compared with EETC-treated groups (Table 3). No significant effect was found on EETC-induced head dipping activity (Table 3).

Effect of haloperidol on the activity of ethanol extract of leaves of *Triumfetta cordifolia* in mice. The effect of haloperidol (0.2 mg/kg, i.p.) on the neurobehavioral activity of EETC (17.5 mg/kg, i.p) is shown Tables 2 and 3. Intraperitoneal administration of haloperidol (0.2 mg/kg) alone significantly ($p < 0.05$) decreased NIR, NIG, head dipping and locomotor activities relative to vehicle control groups (Table 2). However, One-way ANOVA revealed that pretreatment with haloperidol (0.2 mg/kg, i.p.) significantly ($p < 0.05$) enhanced the effects of EETC (17.5 mg/kg, i.p) on NIR and NIG, as both treatments further decrease the rearing and grooming when compared with EETC-treated groups. Moreover, haloperidol significantly ($p < 0.05$) reversed the effect of EETC on locomotor activity (Table 3). Also, there was no significant effect EETC-induced head dipping behavior in mice.

Effect of propranolol on the activity of ethanol extract of leaves of *Triumfetta cordifolia* in mice. The effect of propranolol on the decreased NIR, NIG, head dipping and locomotor activities by EETC (17.5 mg/kg, i.p) is shown Tables 2 and 3. Treatment with propranolol (0.2 mg/kg, i.p.) alone caused a

significant ($p < 0.05$) reduction in NIR, NIG, head dipping and locomotor activities when compared with vehicle control groups (Table 2). Pre-treatment with propranolol (0.2 mg/kg, i.p.) significantly ($p < 0.05$) potentiated the effect of EETC (17.5 mg/kg, i.p) on grooming behaviour; no effects were recorded on NIR and head dipping activity when compared with EETC control group. However, pre-treatment with propranolol (0.2 mg/kg, i.p.) significantly reversed the effects of EETC on locomotor activity in comparison with EETC control group (Table 3).

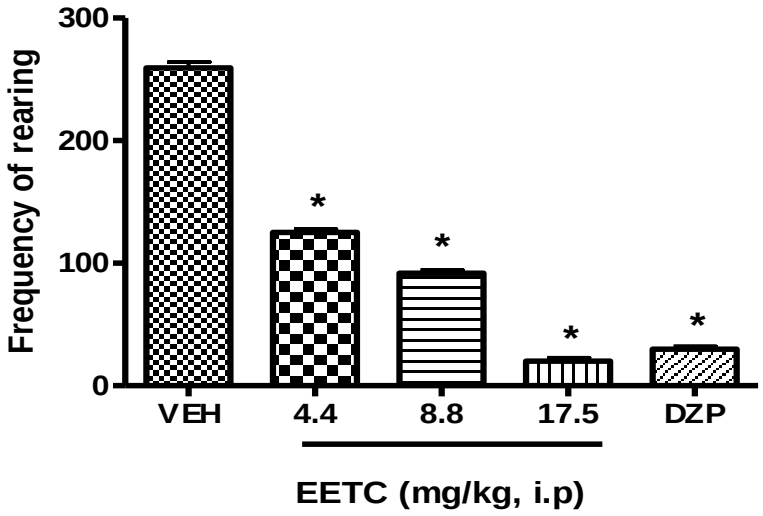

Fig 1. *Effect of ethanol extract of leaves of Triumfetta cordifolia on rearing behavior in mice*
Bars represent the mean of 6 animals / group. *$p < 0.05$ compared to vehicle group (one-way ANOVA followed by Newman-Keuls *post-hoc* test). **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia*, **DZP** – diazepam (2 mg / kg), **NIG –** Novelty-induced rearing.

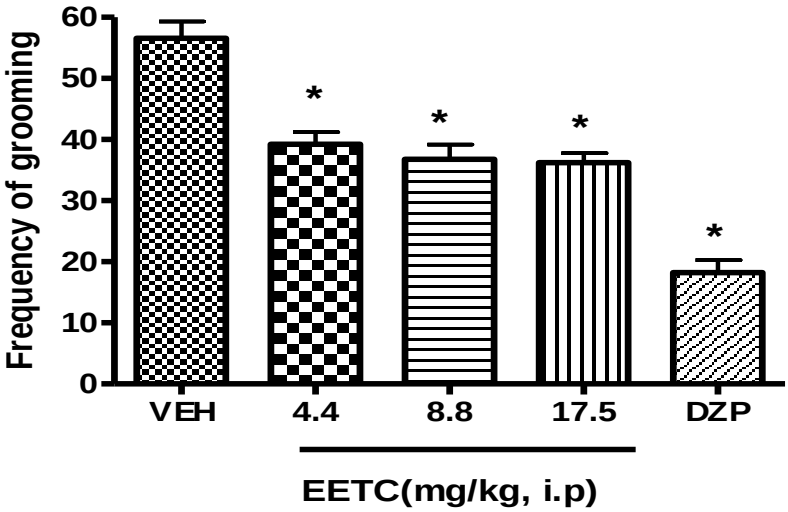

Fig 2. *Effect of ethanol extract of leaves of Triumfetta cordifolia on grooming behavior in mice*
Bars represent the mean of 6 animals / group. *$p < 0.05$ compared to vehicle group (one-way ANOVA followed by Newman-Keuls *post-hoc* test). **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia*, **DZP** – diazepam (2 mg / kg), **NIG –** Novelty-induced grooming.

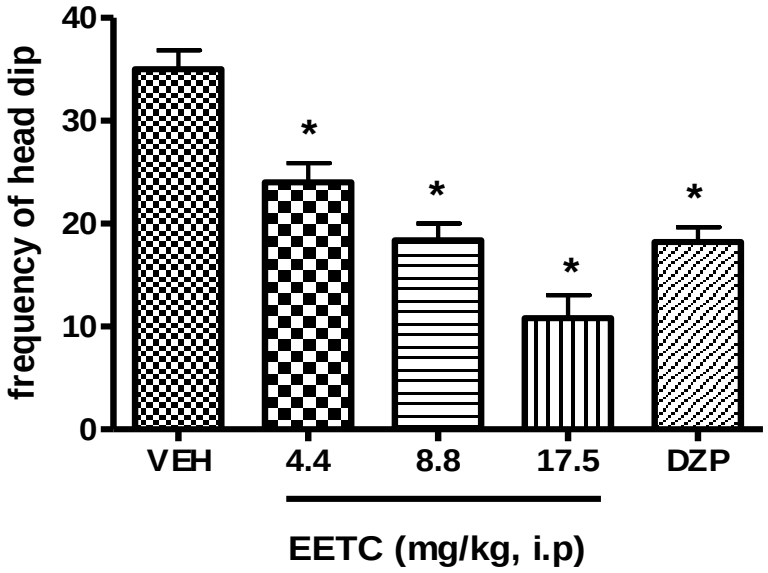

Fig 3. *Effect of ethanol extract of leaves of Triumfetta cordifolia on head dipping activity in mice*
Each bar represent the mean of 6 animals / group. *$p < 0.05$ compared to vehicle group (one-way ANOVA followed by Newman-Keuls *post-hoc* test). **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia*, **DZP** – diazepam (2 mg / kg).

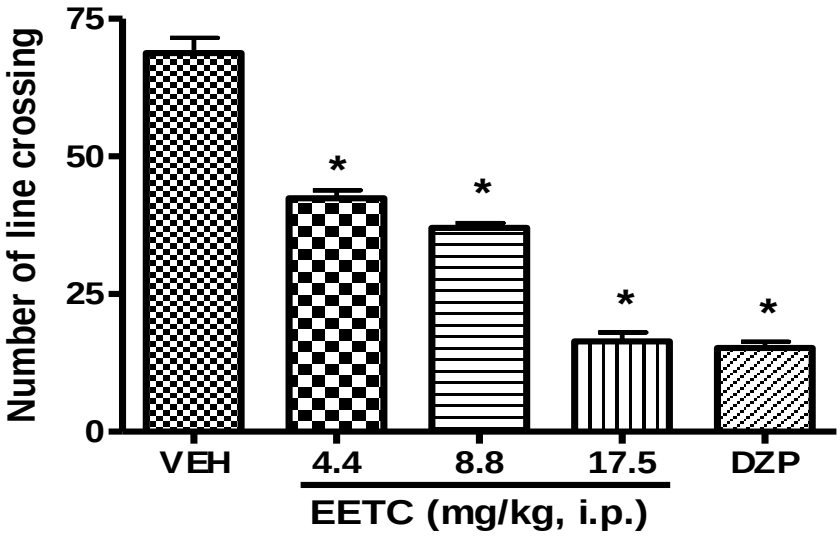

Fig 4. *Effect of ethanol extract of leaves of Triumfetta cordifolia on locomotor activity in mice*
Bar represent the mean of 6 animals / group. *$p < 0.05$ compared to vehicle group (one-way ANOVA followed by Newman-Keuls *post-hoc* test). **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia*, **DZP** – diazepam (2 mg / kg).

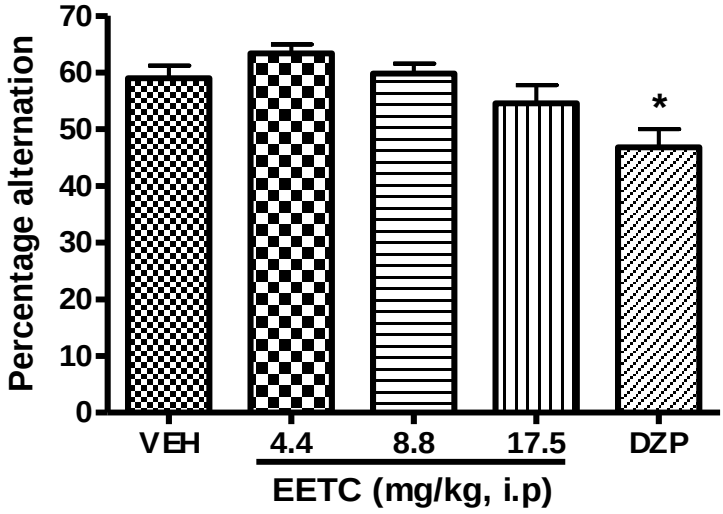

Fig 5. *Effect of the Ethanol extract of leaves of Triumfetta cordifolia on memory performance in mice*
Bar represent the mean of 6 animals / group. *$p < 0.05$ compared to vehicle group (one-way ANOVA followed by Newman-Keuls *post-hoc* test). **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia*, **DZP** – diazepam (2 mg / kg).

Table 1 Effect of the ethanol extract of leaves of *Triumfetta cordifolia* on anxiety-like behavior in mice

Treatment groups	Frequency of open arm entry	Duration in open arm entry	Percentage open arm duration	Index of open arm avoidance
VEH (10 mL/kg)	1.60 ± 0.24	28.00 ± 11.84	12.62 ± 4.24	86.44 ± 4.12
EETC (4.4 mg/kg)	0.25 ± 0.24	8.80 ± 5.43	3.72 ± 2.29	94.22 ± 3.62
EETC(8.8 mg/kg)	0.60 ± 0.24	8.20 ± 3.50	2.88 ± 1.30	93.84 ± 2.74
EETC(17.5mg/kg)	0.60 ± 0.24	8.80 ± 3.83	1.92 ± 1.08	94.86 ± 2.43
DZP (2 mg/kg)	7.00 ± 0.71 *	249.40 ± 3.74 *	85.60 ± 1.75 *	17.00 ± 3.09 *

The results are expressed as Mean ± SEM, (n=6). VEH – vehicle, EETC - ethanol extract of leaves of *Triumfetta cordifolia*, DZP – diazepam (2 mg / kg).

Table 2. Effect of atropine, yohimbine, cyproheptadine, haloperidol and propranolol on novelty-induced rearing, grooming, head dipping and locomotor activities in mice

Treatment groups	NIR/30 min	NIG/30 min	HD/5 min	LA/5 min
VEH (10mL/kg)	259.40 ± 4.67	56.60 ± 2.73	35.00 ± 1.84	68.80 ± 2.71
Atropine (0.5 mg/kg)	128.80 ± 8.42*	40.60 ± 2.56*	27.20 ± 2.56*	67.20 ± 3.43
Yohimbine (0.5 mg/kg)	62.60 ± 4.48*	16.20 ± 0.86*	7.00 ± 0.71*	26.80 ± 1.28*
Cyproheptadine (0.5 mg/kg)	103.00 ± 3.98*	17.20 ± 1.39*	9.40 ± 1.08*	48.20 ± 1.28*
Haloperidol (0.2 mg/kg)	107.60 ± 11.64*	26.00 ± 0.71*	11.00 ± 0.71*	17.00 ± 0.71*
Propranolol (0.2 mg/kg)	96.00 ± 4.04*	42.60 ± 5.42*	21.20 ± 2.22*	23.60 ± 1.21*

Results are expressed as Mean ± SEM, (n=6). *$p < 0.05$ compared to vehicle group. One-way ANOVA followed by Newman-Keuls *post-hoc* test revealed that there is a significant difference between the vehicle and treatment groups. **NIR** – novelty induced rearing, **NIG** – novelty induced grooming, **HD –** Head dip, **LA –** Locomotor activity; **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia.*

Table 3. Effect of atropine, yohimbine, cyproheptadine, haloperidol and propranolol on the neurobehavioral activities of ethanol extract of leaves of *Triumfetta cordifolia* in mice

Treatment groups	NIR/30 min	NIG/30 min	HD/5 min	LA/5 min
VEH (10 mL/kg)	259.40 ± 4.67	56.60 ± 2.73	35.00 ± 1.84	68.80 ± 2.71
EETC (17.5 mg/kg)	20.20 ± 2.70*	22.60 ± 6.65*	10.80 ± 2.25*	13.20 ± 2.62*
Atropine (0.5 mg/kg, i.p) + EETC	23.00 ± 1.48*	8.20 ± 1.46*	11.60 ± 1.21	31.80 ± 1.53**
Yohimbine (0.5 mg/kg) + EETC	9.00 ± 1.52*	2.20 ± 0.58*	13.00 ± 1.00	17.80 ± 2.58**
Cyproheptadine (0.5 mg/kg) + EETC	8.80 ± 1.32*	7.60 ± 1.03*	9.40 ± 1.08	32.00 ± 2.12**
Haloperidol (0.2 mg/kg) + EETC	9.60 ± 1.21*	5.60 ± 0.75*	12.60 ± 0.93	57.00 ± 1.73**
Propranolol (0.2 mg/kg) + EETC	25.0 ± 1.38	11.80 ± 1.11*	13.80 ± 1.02	33.60 ± 0.81**

The results are expressed as Mean ± SEM, (n=5). *$p < 0.05$ compared to vehicle group; **$p < 0.05$ compared to EETC group. One-way ANOVA followed by Newman-Keuls *post-hoc* test revealed that there is a significant difference between treatment and control groups. **NIR** – novelty induced rearing, **NIG** – novelty induced grooming, **HD –** Head dip, **LA –** Locomotor activity; **VEH** – vehicle, **EETC** - ethanol extract of leaves of *Triumfetta cordifolia.*

DISCUSSION

The result of this study revealed that EETC significantly decreased neurobehavioral activities. Specifically, EETC reduced novelty-induced rearing and grooming behaviour in mice. Also, EECT significantly decreased locomotor activity based on reduced number of line crossing in the open-field test. EETC demonstrated sedative behaviour characterized by decreased head dipping activity in the hole board test. However, no significant effect was observed on cognitive performance in the YMT in mice. Also, EETC did not demonstrate significant anxiolytic effect in the EPM relative to vehicle controls.

Nevertheless, mechanistic neural studies using neurotransmitter blockers revealed the involvements of cholinergic, adrenergic, serotonergic and dopaminergic neurotransmissions in the neurobehavioral activities of EETC in mice. Meanwhile, the acute toxicity determination of EETC using the lock's method showed that intraperitoneal administration of EETC produced an LD_{50} of 282 mg/kg body weight. The study showed that not observable adverse effects were observed following 48 h of continuous observation.

The study established the acute toxicity of the crude extract by the determination of LD_{50}. LD_{50} is the dose at which mortality occur in 50% population of the experimental animals. The higher the value of the LD_{50} for a substance, the relatively safe the substance is assumed to be. The LD_{50} determination for the *Triumfetta cordifolia* in mice via the intraperitoneal route was 282 mg/kg body weight. The acute toxicity studies of the ethanol extract of the leaves of *Triumfetta cordifolia* revealed that the leaves exhibit moderate level of toxicity, which was reflected by the low LD_{50} value.

The ethanol extract of the leaves of *Triumfetta cordifolia* decreased NIR in a dose dependent manner. NIR is a behavioral activity of rodent characterized by vertical movement or posture as an excitatory approach to a new environment [18, 19]. It is used to determine the excitatory or sedative activity of psychotropic agents [19]. Drugs that stimulate CNS activity increase rearing behavior whereas those that depress CNS function reduce rearing behavior [20]. In this study, the reduction of NIR activity by EETC suggests the presence of phytochemical compounds that possess sedative activity. Also, reduced NIR by EETC was accompanied by decreased NIG behavior relative to control mice. Grooming is an important behavioral component of rodents that play a deactivating role in restoring homeostasis under stressful situation [21]. It is associated with de-arousal state and decreased CNS activity. Compounds with CNS depressant activity usually inhibit

grooming behavior whereas agents with CNS excitatory potentials increase grooming behavior in animals [22]. However, EETC was found to reduce NIG, suggesting CNS depressant effect and its stress-attenuating role in a novel environment. Besides, we also showed that EETC reduced spontaneous motor activity in the OFT. Previous studies have shown that locomotor activity is mediated by increase in excitatory neurotransmissions like dopamine and glutamate [20, 23]. However, studies have reported that agents with CNS depressant activity decrease locomotor activity probably due to decreased excitatory neurotransmissions [20, 21]. Thus, the ability of EETC to decrease spontaneous motor activity, also suggest CNS depressant effect in mice. Together, these actions may be linked to the ability of the phytochemical constituents of EETC to suppress excitatory neural transmissions such as glutaminergic and dopaminergic systems, or the possible enhancement of central inhibitory systems such as gamma-amino butyric acid (GABA) [13, 22].

Similarly, EETC dose dependently reduced the exploratory behavior, as demonstrated by the reduction of the number of the head dips in the head dip test in mice. The hole board test is a well-known paradigm used as a measure of exploratory behavior that may also reveals the sedative activity of agents [24, 25, 26]. It is also popularly used in the test for anxiety; offering a simple method for measuring the response of an animal to an unfamiliar environment. Accordingly, it can be used for screening of compounds with anxiolytic property [26]. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes that can interfere with intelligence, psychomotor function and memory [27, 28]. The hole board test is based on the assumption that head dipping of the animals is inversely proportional to their anxiety state in a moderately aversive environment [29]. Also, an increase or decrease in exploration of the hole board may reflect a general stimulant or depressant actions respectively. In this study, EETC also produced a dose-dependent decrease in CNS depressant effect, which is devoid of anxiolytic effect. This effect is evidenced by decreased frequency of head dipping behavior in the hole board test, which further suggests the presence of phytochemical compounds with CNS depressant activity. This effect corroborates with the findings of previous investigations, which showed that extracts of *Cissus cornifolia, Ziziphus spina-christi* and *Cryptolepsis sanguinolenta* also produced similar effects related to CNS depressant effects [26,30,31].

Moreover, the elevated-plus maze also revealed that EETC has no anxiolytic effect, as evidenced increased index of open arm avoidance characterized by decreased frequency of open visitation and duration of time spent in the open arm. The elevated plus maze represent one of the most widely used animal models for screening anxiolytic and anxiogenic drugs [32,33]. Previous studies haves shown that anxiogenic drugs tend to increase the number of entries in closed arms and time spent in the closed arms respectively. However, anxiolytic drugs tend to increase the number of entries into open arms time spent in the open arms, suggesting anti-anxiety activity [26]. In this study, we observed that EETC showed no significant anxiolytic effect; as shown by increased index of open arm avoidance characterized by decreased frequency of open arm visitation and decreased duration of time spent in the open arm in the EPM relative to vehicle-treated mice. Also, the test for memory showed that pretreatment with EETC produced no significant effect on memory performance in mice. The Y-maze test is considered to reflect short-term memory and

working memory. It is a simple noninvasive and reliable test for screening of compounds with memory enhancing property and it is based on the ability of rodents to remember correctly the pattern and sequence of arm entries while trying to visit a different arm than the one previously visited [15,20]. However, in this study, the decrease in the percentage correct alternation in the YMT, suggests the absence of memory enhancing property by EETC in mice.

Previous studies have shown that the neurotransmitter hypotheses of neurobehavioral exploration are dependent on the involvement of different neurotransmitter systems such as dopamine, adrenaline, serotonin, acetylcholine etc. [15,17]. Specifically, increased rearing, grooming and locomotor activity have been linked with increased dopaminergic neurotransmission [15,17]. Studies have shown that blockade of dopaminergic receptors by haloperidol, a dopaminergic receptor blocker prevents the actions of test drugs mediating their effects via dopaminergic system, which suggests the role of dopaminergic system in their pharmacological effects [23,34]. Thus, the reversal of the locomotor decreasing effect and the potentiation of NIR and NIG actions of EETC by haloperidol in the OFT suggests that its neurobehavioral effect may be partly mediated via interaction with dopaminergic system; mechanism which might be related to enhancement of central GABAergic activity [22]. Also, yohimbine (α_2-noradrenergic receptor antagonist) and propranolol (β-noradrenergic receptor antagonist) are often used as a research tools for the elucidation of the possible involvement of noradrenergic pathway in the neurobehavioral effects of drugs [35, 36]. Therefore, the blockades of anxiolytic, sedative or antidepressant property of test compounds by adrenergic antagonists (yohimbine, propranolol) serve as a pharmacological probe for the interaction of noradrenergic system [37]. Thus, the finding that the effects of EETC on NIG was potentiated as well reversed by yohimbine and propranolol in the OFT suggests the involvement of central noradrenergic neurotransmission in its neurobehavioral effect in mice.

Experimental and clinical studies have also revealed the involvement of serotonergic (5-hydrotryptaminergic, 5-HT) system in neurobehavioral activity such as mood elevation and sleep systems [38], and modulation of serotonergic pathways underlie the therapeutic effect of psychotropic drugs [38]. Thus, antagonism of the effects of drugs by cyproheptadine, a non-selective 5-HT_2 receptor antagonist is an indication that the compounds may be mediating its action via interaction with serotonergic system [38]. In line with perspective, the interaction studies involving EETC with the serotonergic receptor antagonist, cyproheptadine significantly reversed the effect of EETC on locomotion in the OFT. Moreover, pretreatment with cyproheptadine also synergistically potentiated the effects of EETC on NIR and NIG in the OFT in mice, which suggests modulation of central serotonergic neurotransmission in the neurobehavioral effects of EETC in mice. Similarly, atropine (muscarinic cholinergic antagonist), at a dose which does not promote a significant effect *per se* cholinolytic effect, was able to reverse the effect of EETC on locomotion and enhanced EETC-induced decreased grooming behavior, although there was no significant effect on the anti-rearing and sedative effects of EETC. This result suggests that EETC-induced neurobehavioral changes observed in the OFT, may also involve, at least in part, an interaction with cholinergic system [17].

In conclusion, ethanol extract of the leaves of *Triumfetta cordifolia* decreased rearing, grooming and locomotor activity, enhanced memory performance and produced sedative effect, although did not show significant

anxiolytic effect in mice. These effects may be related to the modulations of neurotransmitters such as dopamine, noradrenerline, serotonin and acetylcholine.

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