

**ANXIOLYTIC-LIKE EFFECT OF CRUDE METHANOL EXTRACT OF
ERYTHROPHLEUM IVORENSE IN MICE**

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ABSTRACT

Erythrophleum ivorenses A. Chev (Fabaceae) is a medicinal plant used in folk medicine in the management of pain, swollen condition, antiemetics and also in treatment of convulsive disorder. No existing data was found on the neurobehavioural properties; hence, we examined the modifications of behavioural indices evoked in mice by the introduction of methanol extract from stem bark of *Erythrophleum ivorense* under the conditions of four neurobehavioural tests. Using absolute methanol, the plant was macerated and the crude methanol extract (CME) was obtained. Intraperitoneal injections of CME at doses 5, 10, or 20 mg/kg and diazepam (1 mg/kg) or control (10ml/kg) were used; the effects observed were compared with the action of the control. In the hole-board test, injections of the CME significantly ($p < 0.05$) increased in a dose-dependent manner in the manifestations of research behaviour (increased the number of head dips into openings) as compared with control. In open field test, CME significantly reduced rearing in a dose dependent manner while, grooming significantly ($p < 0.05$) reduced at the maximum dose (20 mg/kg) as compared with controls. Administration of CME in dark and light experimental model greatly increased the time spent and the number of entrance of mice into light compartment. In elevated plus-maze CME treated mice exhibited significant increase ($P < 0.05$) in the number of open arm entries, time spent in open arm, but decrease in time spent in closed arm compared with control. In order to establish the mechanism involved in the neurobehavioural effect, flumazenil a receptor antagonist for GABA receptor was pretreated and the results indicated that flumazenil (2.0 mg/kg, i.p.) blocked the NIR inhibition induced by the methanol extract. The results obtained suggest that the methanol stem bark extract of *E. ivorenses* possesses neurobehavioural effect via GABAergic pathway and may account for its use in ethnomedicine.

KEYWORDS: *Erythrophleum ivorenses*.

INTRODUCTION

Anxiety is a horrible condition of inner turmoil which is often associated with nervous behaviour, somatic complaints and rumination.^[1] When there is excessive anxiety, the situation may be regarded as anxiety disorder and this can seriously reduce the standard of life bringing about a number of psychosomatic diseases.

Anxiety disorder afflicts approximately 10% of the world population and is classified as panic attacks, social phobias or generalized anxiety disorders.^[2] It is portrayed by extreme or irrational fear connected with a real or anticipated stimulus. It is commonly associated with phobic avoidance and a constellation of somatic symptoms.

Phobic avoidance may be considered as an adaptive mechanism that permits the individual to reduce exposure to circumstances that may provoke anxiety. But

such avoidance can turn out to be maladaptive when it results in notable behavioural changes, encompassing social isolation and agoraphobia.^[3] Many animal anxiety models examine the natural behavioural patterns of mice and rats to enhance ethologically based behavioural tasks.^[4] These consist of 'approach-avoidance' tasks^[5] in which animals are subjected to an aversive/ threatening environment. For instance, open, elevated arms of the elevated plus-maze, light arena (light/dark exploration/emergence tests) and open field tests with anxiety-like behaviour (phenotype) in each case, inferred from increased avoidance.

Other models include social interaction tests^[6], punishment-based conflict procedures (e.g. punished drinking^[7], defensive burying tests^[8], predator stress^[9], and the examination of ultrasonic vocalizations induced by stress such as maternal separation^[10], while novel techniques include the use of radiotelemetry to assess a

variety of physiological parameters in real time (e.g. core body temperature).^[11]

In recent years, several types of herbal medicines have been put to use as anxiolytic drugs in different parts of the world. The essential oil of *Stachys lavanduifolia* has been reported to possess anxiolytic effects with comparatively lower sedative activity than diazepam.^[12]

Erythrophleum ivorense (A Chev, family, Fabaceae) is a large evergreen tree widely distributed in tropical regions of Western Africa, spreading from Gambia to the Central African Republic and Gabon. With the capacity to grow up to 40 m high and source of hard-heavy wood, the plant is among the highly utilized timber trees in West Africa. The diameter is usually 60–90 cm.^[13] The plant known in native countries by its local names, including “Epo obo” among Yoruba people of South Western Nigeria and “potrofum” among the Akans in Ghana is also widely traded for medicinal uses. In traditional African medicine, stem bark of *Erythrophleum ivorense* is useful in the treatment of pain, swollen, emetic and convulsive disorder.^[13]

The bark of *Erythrophleum ivorense* is traded as ‘sassy-bark’, ‘man cona bark’, ‘casca bark’ or ‘ecorce de tali’ and has quite a lot of medicinal uses.^[14] Stem Bark extracts of *Erythrophleum ivorense* are used orally in Sierra Leone as an emetic and purgative and is externally used to alleviate pain.^[15] According to Betti (2004)^[16], *Erythrophleum ivorense* is orally taken as a laxative and outwardly to ease pains. The bark and sometimes the seeds are generally employed as hunting and ordeal poison.^[17] In Liberia and Garbon, there is preference for the bark of *Erythrophleum ivorense* over *Erythrophleum suaveolens*.^[18]

Erythrophleum ivorense is found dispersed in evergreen primary and secondary forest and moist semi-deciduous forest.^[19] Pieces of root or bark are employed as a protective and as love charm.^[13] Crude methanol extract of *E. ivorense* was reported to have anticonvulsant, sedative, antinociceptive, and antiinflammatory activities.^{[20][21]}

In other research group, methanol extract of *E. ivorense* revealed its anti-microbial and cytotoxic effects.^[22] Also, compound obtained from *Erythrophleum ivorense* root-back demonstrated anti-inflammatory property.^[23] Antioxidant and antimicrobial properties of *Erythrophleum ivorense* was recently reported.^[24] Secondary metabolites extracted from plants have been utilized as sources of derivatives with a large spectrum of biological activities.^[25] Screening of literature revealed that plant samples of *E. ivorense* have been previously examined for their chemical constituents. However, among the numerous phytochemicals recognized to be present in *E. ivorense* are alkaloids which include: cassaine, cassamidine coumidine, cassaidine, erythrophillamine and erythrophleguine 19-

hydroxycassaine, norcassaide, norcassamide and norerythrophlamide^{[26][27]} and terpenoid, especially diterpene was detected from its root back.^[23]

MATERIALS AND METHODS

Plant material

Identification, collection and authentication of plant materials.

Fresh stem bark of *Erythrophleum ivorense* (*E. ivorense*) was identified and collected from trees in Iwo in Iwo Local Government area of Osun State, Nigeria between the months of April and October 2014. The plant was authenticated by a Botanist in the Department of Botany, Obafemi Awolowo University Ile-Ife, where voucher specimen was deposited (voucher number 16878).

Extraction of plant material

Extraction of plant material followed the methods of Wadood *et al.* (2013).^[28] *Erythrophleum ivorense* stem bark weighing 2.5 kg was air-dried for eight weeks and reduced to coarse powder using an electric blender (Christy and Norris – 47362, England). Extraction was performed by adding *Erythrophleum ivorense* stem bark powder to 5 liters of absolute methanol in a sterile flask with a stopper (to prevent loss of volatile liquid); the mixture was extracted by agitation. After 24 hour, it was decanted; and filtered using filter paper No. 1 (Whatmann London, UK).

The filtrate was evaporated to dryness using a rotary evaporator (Buchi Rota Vapour R110) and freeze-dried until a solid mass was obtained. The dried residue (85.6 g) was sealed tightly in glass vials and stored in a refrigerator at 4° C until use.

Animal materials

Healthy male Swiss mice (20-30 g) obtained from the Animal house of the Ladoke Akintola University of Technology, Ogbomosho, Oyo State Nigeria were used for this study. Animals were housed in standard cages at six animals per cage. General housing was in a temperature-controlled (22.5°C ±2.5°C) quarters with light on/off at 7 o'clock. Mice were given free access to food and water except during behavioural tests.

All animals were fed with commercial standard rodent chow (calories: 29% protein, 13% fat, 58% carbohydrate) all through the experimental period. All rules applying to animal safety and care were observed.

Hole board test

The hole board test^[29] was adopted in this test. It was made of gray Perspex. The LETICA board (signo 720; Printer LE 3333) of dimensions 40 cm × 40 cm, containing 16 evenly spaced holes (3 cm diameter and 2.2 cm depth), with in-built infra-red sensors was used for the study. The matt finishing of the upper panel prevented reflections which may alter the animal behaviour.

An animal was put in the middle of the hole board and permitted to explore the apparatus without restrictions for 5 min. The number of times an animal dipped its head into the holes was counted and recorded automatically by the instrument.^[30]

Elevated plus-maze test

Activity and anxiety-related behaviours were evaluated using the mouse elevated plus-maze (EPM) test as portrayed by Dawson and Tricklebank (1995) with slight modifications. The apparatus contained two open and two enclosed horizontal perpendicular arms (25 × 5 cm) positioned 30 cm above the floor.

The junction of four arms formed a central square platform (5 × 5 cm). The experiments were performed in a lighted and quiet laboratory room. The environment was illuminated by two 60 W white fluorescent lights placed 3 m away from the EPM. Mice were pretreated with extract (5, 10, 20 mg/kg i.p) or diazepam (1 mg/kg i.p) for 30 minutes before submitting them to the EPM apparatus.

Each animal was put in the middle platform facing one of the open arms and permitted to explore freely for 5 min. The maze was carefully cleaned between each trial with 10% ethanol solution and afterwards, by a dry cloth. During the 5 min period, the behavioural parameter of each mouse was recorded as: the number of entries into the open or closed arms (OAE) or (CAE) and average time spent by mouse in each of the arms (OAET), (CAET). The number of entries into closed arms (CAE) is an indication of animal activity.

The mice were pre-treated with flumazenil (2.0mg/kg, i.p.) 30 minutes prior to the administration of the CME (5.0, 10, and 20 mg/kg, i.p.) and diazepam (1.0 mg/kg, i.p.).

Light and dark test

The light and dark apparatus comprised open top wooden box. Two separate chambers, a black chamber (25 cm long × 35 cm wide × 35 cm deep) painted black and made dark by covering its top with black plywood, and a bright chamber (25 cm long × 35 cm wide × 35 cm deep) painted white and brightly illuminated with 25-W white light source was placed 25 cm above the open box.

The two chambers were linked through a small open doorway (7.5 cm long × 5 cm wide) placed on the floor level at the middle of the partition. The animals were permitted to explore the light-dark arena 30 min after receiving the extract (5, 10, 20 mg/kg i.p) or diazepam (1 mg/kg i.p) as a standard anxiolytic compound.

The behaviour of each mouse was then tracked for 5 min and the number of entrance into the light compartment and the time spent in the light compartment of the apparatus were measured. An increase in the mentioned

parameters was regarded as the anxiolytic effect of compound.

Novelty-induced rearing (NIR) and grooming behaviour

The open field test is a commonly utilized model of anxiety-like behaviour adopted to assess emotionality in animals and is based on exposing an animal to an unfamiliar environment whose escape is precluded by surrounding walls. A standard set was used for the open field test on mice.

A rectangular arena with a hard floor (36×36×26 cm with 26cm walls) was made up of white painted wood. The arena was divided by permanent red markings into 16 equal squares. Spontaneous motor activity was monitored for 30 min.^[31] Animals were pretreated with the vehicle (10 ml/kg), diazepam (1 mg/kg i.p) or extract (5, 10, 20 mg/kg i.p) for 30 minutes before exposure to the open field paradigm.

Behaviours were assessed as described by Ajayi and Ukponmwan (1994).^[31] Each mouse was put into the field, and the rearing frequency (number of times the animal stood on its hind limbs, with four limbs against the walls of the observation chamber or free in air) and frequency of grooming (number of body cleanings with the paws, picking of the body and pubis with the mouth, and face “washing” episodes) were recorded within 10-min-long observation periods.

The arena was then cleaned with 70% alcohol to eliminate olfactory bias and permitted to dry before introduction of another animal. Apparatus was cleaned carefully between trials with water. All behavioural recordings were performed with the observer unaware of the treatment the mice had received.

Statistical analysis

All results are expressed as mean±standard error of the mean. The data were analyzed statistically using one way analysis of variance ANOVA, followed by the Newman-Keuls post hoc test for multiple comparisons. P<0.05 was taken to be statistically significant. Results were presented as tables.

RESULTS

Effects of CME of *Erythrophleum ivorense* stem bark extract on novelty-induced rearing and grooming.

There were significant differences between mice treated with vehicle (10 ml/kg, i.p.) and the mice that were treated with the extract (5, 10, and 20 mg/kg, i.p.) as well as those that received diazepam (1 mg/kg, i.p.) (Fig. 1). Administration of CME (5-20 mg/kg i.p) significantly reduced rearing in a dose-dependent manner while, grooming was significantly reduce at a dose of 20mg/kg and almost the same with the standard (figure 1). The inhibition of novelty-induced rearing by the CME was reversed by flumazenil, a GABA_A and benzodiazepine specific antagonist. The reversal of methanolic extract-

induced inhibition of NIR by flumazenil was significant ($p < 0.05$) at the maximum dose of the extract (Table 1).

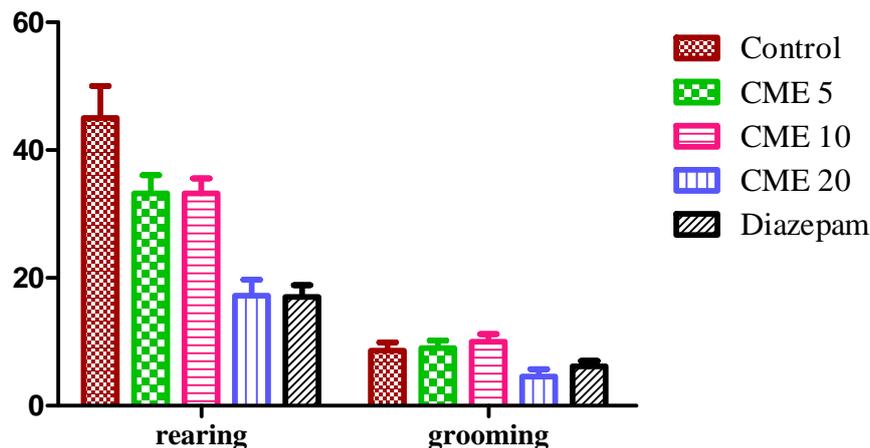


Figure 1: Effect of CME of *Erythrophleum ivorense* on novelty-induced rearing and grooming. Each column represents the mean±SEM (n=5 per group).

* $P < 0.05$ compared to control group. ANOVA followed by Newman-Keuls Multiple Comparison tests

Table 1: Effects of flumazenil (2mg/kg, i.p) on inhibition of rearing induced by CME in mice.

Pretreatments	doses (mg/kg)	rearing behavior**
Control	0	70.2±2.13
Flumazenil	2	65.7±3.57
CME	5	65.1±1.42
CME	10	64.7±0.97
CME	20	23.5±2.53*

**Values are recorded as means±SEM (n=5).

*Values are statistically significant ($p < 0.05$) in relation to control. One way ANOVA followed by Newman-Keuls Multiple Comparison tests

Light and Dark Box

Treatment with diazepam greatly increased the time spent in light box and the number of entrance ($P < 0.05$) into the light boxes; whereas the time spent in dark box and number of entrance were significantly reduced. *Erythrophleum ivorense* extract treated mice also showed

significant increase in the time spent and the number of entrance into light compartment. However, the time spent in and number of entrance into the dark compartment were significantly reduced ($P < 0.05$) as compared to the vehicle treated group (Table 2).

Table 2: Effect of intraperitoneal administration of CME of *Erythrophleum ivorense* stem bark extract or diazepam on the behaviour of mice in Light–dark test.

Treatments	Doses (mg/kg)	Time spent in the boxes(s)**	Entrance number**	Dark box lighted
Vehicle	0	120.5±5.03	167.3±9.21	17.01±3.2
Diazepam	1	177.5±8.01*	110.5±1.23*	25.05±1.76
CME	5	196.3±7.96*	132.7±8.73*	27.71±0.87
CME	10	241.5±6.18*	129.3±4.65*	28.12±1.33
CME	20	251.6±7.16*	105.1±7.71*	31.53±5.61

**Values are recorded as means±SEM (n=5).

*Values are statistically significant ($p < 0.05$) in relation to control. One way ANOVA followed by Newman-Keuls Multiple Comparison tests

Elevated Plus-Maze

Diazepam treated mice showed significant increase ($P < 0.05$) in the number of open arm entries and the time spent in open arms. They indicated a decrease in the time spent in closed arm [Table 3]. CME of *Erythrophleum*

ivorense stem bark extract (5, 10 and 20 mg/kg) treated mice exhibited significant increase ($P < 0.05$) in the number of open arm entries, time spent in open arm, but decrease in the time spent in closed arm compared with control.

Table 3: Effect of CME on behaviour of mice in Elevated Plus-Maze.

Treatments	Doses (mg/kg)	Time spent in the arms (s)**	Open arm entries	open arm closed
Vehicle	0	60.5±3.07	135.3±4.21	45.1±0.34
Diazepam	1	65.5±4.01	130.0±2.01	56.4±1.09
CME	5	96.9±1.43	121.3±2.65	55.9±2.13
CME	10	121.7±2.05	112.7±1.35	61.5±1.71
CME	20	127.3±3.45	98.1±6.32	81.7±3.41

**Values are recorded as means±SEM (n=5).

*Values are statistically significant (p<0.05) in relation to control. One way ANOVA followed by Newman-Keuls Multiple Comparison tests

Hole board test

CME of *Erythrophleum ivorense* stem bark extract (5, 10 and 20 mg/kg) treated mice greatly increased the number

of head dipping as compared to the control animals (fig 1). CME at doses 5 and 10 mg/kg were almost the same with the standard drug (diazepam).

Hole board test

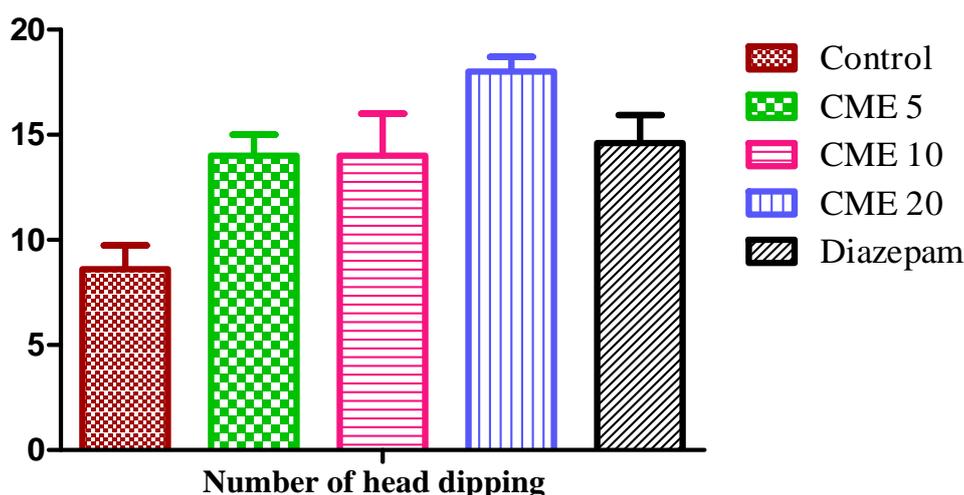


Figure 1: Effect of Erythrophleum ivorense extract (CME) on head dipping in mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

DISCUSSION

This study was designed to assess the effect of intraperitoneal administration of methanolic extracts of *Erythrophleum ivorense* in mice during locomotion, anxiety and fear tests using open field, elevated plus-maze and light and dark experimental models.

The results of the studies provided evidence that the methanolic extract of *Erythrophleum ivorense* stem bark possesses a wide spectrum of CNS activity. In open field test, locomotor activity is regarded as an index of alertness and a decrease in it is indicative of sedative activity.^[32] The effect of CME significantly decreased the locomotive activity of mice indicating sedative effect which supported our earlier finding on anticonvulsant and sedative activities of CME in mice.^[20]

Flumazenil a receptor antagonist for GABA receptor was pretreated to establish the receptors involved in the observed modulation of NIR by the extract.^{[33][34]} The study indicated that flumazenil (2.0 mg/kg, i.p.) blocked

the NIR inhibition induced by the methanol extract. This may shows that crude methanolic extract may be exhibiting its NIR inhibitory activity via GABA (Table 1). GABA is known to participate in locomotor activity in rodents as one of the inhibitory neurotransmitters. Stimulation of the inhibitory GABA receptors was reported to induce sedation.^[35] The influence of flumazenil in this experiment may suggests that the extracts of *Erythrophleum ivorense* exhibited its sedative effect in mice through GABAergic neural system.

The elevated plus-maze (EPM) is regarded to be an etiologically valid animal model of anxiety because it utilizes natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can provoke anxiety in humans. An anxiolytic agent increases the time spent in open arms and decreases the time spent in enclosed arm of the EPM.^[36] In the present study, intraperitoneal administration of *Erythrophleum ivorense* stem bark extract demonstrated an anxiolytic-like effect in mice, as it greatly increased the time spent

in open arms and reduced the time spent in closed arms. The anxiolytic effect of the extract was further confirmed by the findings of the light and dark box. It is employed to assess an ethological-based approach-avoidance conflict test and very sensitive to drugs that affect anxiety. In this test, the number of transitions between the light and dark compartments and the time spent in the light side are identified as anxiety indices, despite the transition parameter being highly dependent on locomotor activity.^[37] Mice treated with *Erythrophleum ivorense* showed increase in the time spent in the light compartment and decrease in the numbers of shuttle crossings, validating the activity upon the main anxiolytic parameter.

The hole board test is used to model anxiety in animals. In this test, an anxiolytic-like state reflected an increase in head-dipping behaviours.^{[38][39]}

Our results showed that methanolic extract of *Erythrophleum ivorense* increased the head dipping, corroborating the anxiolytic-like effect previously shown in the light- dark test.

In conclusion, the results obtained suggests that the methanol stem bark extract of *E. ivorense* possesses neurobehavioural effect via GABAergic pathway and may account for its use in ethnomedicine.

Declarations

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Competing interests

The authors declare that they have no competing interest.

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