

Behavioural, Sedative and Anticonvulsant Activities of Ethanol Extract of the Leaf of *Lagenaria breviflora* (Benth) in Mice

A. I. OLURANKINSE^{ABE}, J. O. IMORU^{*BCDEF}, I. A. OYEMITAN^{ACEF}

Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

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

Abstract

Background: *Lagenaria breviflora* Benth is used in West Africa as a traditional remedy for the treatment of gastrointestinal disorders, the treatment of human measles, digestive disorders, and as a wound antiseptic.

Objectives: This study investigated the behavioural, sedative, and anticonvulsant activities of the ethanol extract of *Lagenaria breviflora* (EOLB) in mice, and its possible mechanism(s) of action.

Materials and Methods: The CNS activities of EOLB (100, 200, and 400 mg/kg, *i.p.*, n=6) were evaluated using animal models of novelty-induced behaviours (NIB), sedation (ketamine-induced hypnosis), and anticonvulsion (maximal electroshock (MES), pentylenetetrazole (PTZ), and strychnine). Probable mechanism(s) were evaluated using an antagonist (flumazenil, 2 mg/ml, *i.p.*) and an agonist (amphetamine (2 mg/kg, *i.p.*) on NIB.

Results: LD₅₀ values obtained were 1225 mg/kg and greater than 5000 mg/kg for the intraperitoneal and oral routes respectively. The EOLB at all doses tested significantly (p<0.01) inhibited the NIB. The EOLB (400 mg/kg) significantly (p<0.01) shortened sleep latency and significantly (p<0.05) prolonged the total sleeping time induced by ketamine (100 mg/kg, *i.p.*). EOLB at 400 mg/kg gave 33.3 % protection against HLTE caused by the MES-induced convulsion, significantly (p<0.01) prolonged the time of death, and offered 83.3 % protection against convulsion caused by PTZ. Flumazenil and amphetamine (2 mg/kg, *i.p.* respectively) significantly (p<0.001) reversed the effects of the EOLB on NIB.

Conclusions: The study concluded that the ethanol leaf extract of *Lagenaria breviflora* is safe orally and mildly toxic intraperitoneally. The extract showed significant depressive activity on the central nervous system, sedative and anticonvulsant activities which may be mediated through augmentation of GABAergic or inhibition of the dopaminergic neural pathways, thus supporting some of the tradomedicinal claims of the uses of *Lagenaria breviflora*.

Keywords: *Lagenaria breviflora*; LD₅₀; Novelty-induced behaviour; Anticonvulsant: Maximal electroshock; Pentylenetetrazole

INTRODUCTION

The traditional medicine system has used plants for thousands of years in countries such as India and China. These plant-based systems have continually played crucial roles in health care (Ogundaini, 2005). Several commonly used drugs today are of herbal origin (Fabricant & Farnsworth, 2001). *Lagenaria breviflora*, known as *Tagiri* in the Southwest, *Gojinjima* in the North, *Ogbenwa* in the East (Igbo) parts of Nigeria (Burkill, 2004), belonging to the family Cucurbitaceae, is a perennial climber with a herbaceous stem which may be up to 6 m long. The stem scrambles over the ground or climbs into the surrounding vegetation, attaching itself by means of tendrils. Usually, it is gathered from the wild for local use as medicine (Oridupa and Saba, 2018).

METHODOLOGY

Materials

Plant collection and identification

The leaf of *L. breviflora* (Benth) was identified and authenticated by Mr. I. I. Ogunlowo of the Pharmacognosy Department. Thereafter fresh *Lagenaria breviflora* leaf was obtained from the wild along Laje Road, Ondo town, Ondo State. Herbarium specimen of the leaf was deposited at the Herbarium (FPI: 2256), Pharmacognosy Department, Faculty of Pharmacy, OAU, Ile- Ife.

Equipment and reagents

Weighing balance (Metler Toledo) Top loading balance, animal cages, needles and syringes (1ml, 2mls, 5mls), electroconvulsimeter, sample bottles, distilled water, stopwatch, plexiglass, mortar, and pestle.

Drugs

Diazepam (Valium, Roche, Switzerland), Pentylentetrazole (PTZ), Strychnine, Ketamine, Amphetamine, Flumazenil (Aladdin F124794-25) and Sodium valproate.

Experimental animals

Adult Albino mice (18-22 g) obtained from the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife were used for the research. They were kept under a

Lagenaria breviflora Benth is used in West Africa as a traditional remedy for the treatment of gastrointestinal disorders, the treatment of human measles, digestive disorders, and as wound antiseptics e.g., umbilical cord wounds (Adeolu *et al.*, 2013). Ethanol leaf extract of *Lagenaria breviflora* may possess anti-inflammatory as well as analgesic properties, (Adeolu *et al.*, 2013).

In spite of the fact that *L. breviflora* is used in traditional medicine, little is known regarding the sedative and anticonvulsant properties of the leaf, hence this study investigated the behavioural, sedative, and anticonvulsant activities of the ethanol extract of *L. breviflora* (EOLB) in mice, and its possible mechanism(s) of action.

conducive laboratory condition (Olayiwola *et al.*, 2013) and fed with standard animal feed prior to and throughout the period of the experiment.

Institution approval

The research was conducted under the internationally accepted principles for laboratory animal use and care as found for example in the European community guidelines (EEC Directive of 1986; 86/609/EEC). All experiments were examined and approved by the Faculty of Pharmacy board committee and Postgraduate board committee of Obafemi Awolowo, University Ile-Ife, Nigeria.

Methods

Preparation of extract

The fresh leaf *L. breviflora* was air-dried before being milled into powder with the aid of an electric grinder. About 2000 g of the powdered leaf of *L. breviflora* was packed into a round-bottom flask and soaked with 80 %v/v of ethanol. The flask was then mounted on a mechanical shaker for 3 days after which it was poured into a muslin bag for filtration. The filtrate was concentrated into a solid paste using a rotary evaporator at a maximum temperature of 45°C to obtain the crude ethanol extract of the plant. The solid paste of the ethanol extract of the plant was then stored in the refrigerator until it was needed for use. The crude ethanol extract was subsequently dissolved in distilled water for administration.

Administration of drugs

For the determination of the oral route lethal dose (LD₅₀) of the extract of *L. breviflora* an oral cannula was used. Strychnine and diazepam were dissolved and diluted respectively in distilled water (normal saline (0.9% NaCl), while Pentylene-tetrazole was also dissolved in distilled water. Intraperitoneal (i.p) route. The extract of *L. breviflora* was administered intraperitoneally for the determination of the intraperitoneal lethal dose in mice. The *i.p.* route was utilized for the behavioural, sedative, and anti-convulsion experiments.

General experimental design

Animals were randomly divided into 5 groups (n=6). Group I served as the negative control and received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with the extract at doses of 100, 200, and 400 mg/kg respectively, while group V, the positive control group received the appropriate standard drug.

Determination of the LD₅₀

The method described by Lorke (1983) was used to determine the LD₅₀ values. This model involves two phases. In the first phase, three increasing doses of 10, 100 and 1000 mg/kg of the extract were administered intraperitoneally or orally to three different groups of mice (n=3). In the second phase, 400, 600, 800 and 1000 mg/kg of the oil were administered intraperitoneally to four groups of mice (n=1) and four dose levels of 1000, 1600, 2900 and 5000 mg/kg of the oil were administered orally to four groups of mice (n=1). The mice were observed closely for 60 min for immediate effect and mortality within 24 h of treatment was recorded.

Mathematically, the LD₅₀ = $\sqrt{A \times B}$

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

Effect of *Lagenaria breviflora* on Novelty-Induced Behaviours (NIB)

Open Field Model (OFM) as described (Onigbogi *et al.*, 2000) was used to test novelty-induced behavioural activities of rearing (which is the number of times the animal stands on its hind limbs with forelimbs raised in the air or from its forelimb against the wall of the observation box), grooming (nose and face wash, mouth cleaning) and locomotion (number of Squares crossed with all the limbs on the floor of the observation cage) in mice.

The mice were randomly divided into five groups (n=6). Each mouse in group 1 received 10 ml/kg of distilled water as negative control intraperitoneally (*i.p.*). Groups 2, 3 and 4 were administered with different doses: 100, 200 and 400 mg of extract of *Lagenaria breviflora* respectively via intraperitoneal route and group 5 received diazepam (2 mg/kg) to serve as the positive control. All the mice were pre-treated for 30 mins with the vehicle, test materials and standard drug prior to assessment for rearing, locomotion and grooming for a period of 20 mins.

The mice were placed directly from the home cage into an opaque plexiglass observation cage (45 x 25 x 25 cm) with only one side transparent for observation. All the mice were observed and assessed singly, used only once, and the plexiglass was sanitized with 70% alcohol after each assessment to remove olfactory cues from one animal to the other (Blanchard *et al.*, 2001). The frequency of the episodic rearing and locomotion was quantified by using a statistical tally counting and a stopwatch for 20 min. Test materials: distilled water (10 ml/kg, *i.p.*) as the vehicle, extract of *L. breviflora* 100, 200, and 400 ml/kg and diazepam (2 mg/kg) doses were administered intraperitoneal (*i.p.*)

Effect of *Lagenaria breviflora* on ketamine-induced hypnosis

The test was done using the methods described by Mimura *et al.*, 1990 in order to determine the effects of the extract of *Lagenaria breviflora* on sleep latency and prolongation of total sleeping time induced by the ketamine. Thirty mice were randomly selected and allotted into five groups (n=6). Group 1 was administered distilled water (negative control). Groups 2-4 were injected with 100, 200, and 400 mg/kg *i.p.*, of the extract of *Lagenaria breviflora* respectively. Group 5 was injected with 2 mg/kg, *i.p.* of diazepam (positive control). After 30 min pretreatment of the mice, ketamine (100 mg/kg, *i.p.*) was administered. The time interval between ketamine administration and loss of righting reflex was taken as latency to sleep, while the time from the loss to the recovery of the righting reflex, was the duration or total sleeping time (TST) (Rabbani *et al.*, 2003).

Effect of *Lagenaria breviflora* on chemo- and electroshock-induced convulsion

The anticonvulsant potential of the extract of *Lagenaria breviflora* was assessed using chemo-convulsant (pentylene-tetrazole and strychnine) and electroconvulsion maximal-electroshock, (MES) models.

Effect of Lagenaria breviflora on PTZ-induced convulsion

The convulsion model as induced by Pentylene-tetrazole (PTZ) (Swinyard *et al.*, 1989) was used. Thirty-nine mice were randomly allocated into five groups (n=6). Group 1 was administered distilled water (negative control). Groups 2-4 were treated with 100, 200 and 400 mg/kg; *i.p.* of extract of *L. breviflora* respectively, while Group 5 was administered with 2 mg/kg diazepam (positive control). All groups were pre-treated 30 min prior to administration of PTZ (85 mg/kg *i.p.*). Each treated mouse was assessed for onset of convulsion or convulsion Latency (CL) and time of death (TD) for 30 mins. Percentage protection was calculated for each treated group and compared to the control (vehicle and reference drugs). Animals that survived beyond 30 mins post-PTZ injections were assumed in this model as protected (Amabeoku and Kinyua, 2010) and the time of death was assumed to be 30 min for animals that survived beyond 30 mins for the purpose of statistical analysis. The CL and TD were expressed as mean \pm SEM and statistically compared to the vehicle group. Protection was calculated as the ratio of animals that survived to the number of animals in each group.

Effect of extract of Lagenaria breviflora on strychnine-induced convulsion

Strychnine is a known direct glycine antagonist in the spinal cord and brain stem (Curtis *et al.*, 1971). The procedure uses strychnine STR 4 mg/kg *i.p.* as the convulsant agent (Swinyard *et al.*, 1989). All the test materials (distilled water; EOLB and diazepam 2 mg/kg, *i.p.*) were administered intraperitoneal 30 mins before injection with strychnine. Each treated mouse was assessed for onset of convulsion or convulsion latency (CL) and time of death (TD) for 30 mins. Percentage protection was calculated for each treated group and compared to controls (vehicle and reference drugs). Animals that survived beyond 30 min post-STR injection were assumed in this model as protected (Amabeoku and Kinyua, 2010), and the time of death was assumed to be 30 min for animals that survive beyond 30 min for the purpose of statistical analysis. The ratios of animals that survived to the number of animals in each group were also estimated and expressed in percentage. The abolition of tonic extensor jerks of the hind limbs was considered an indicator that the test agent materials could prevent strychnine-induced convulsion in mice.

Effect of Extract of Lagenaria breviflora on electroshock-induced convulsion

The effect of the extract of *L. breviflora* on generalized seizures was evaluated by the maximal electroshock, MES method (Swinyard *et al.*, 1989). Generalized seizures were induced with electroshock through ear lobes by electrode clamp which delivered on an alternate current of shock duration, frequency and pulse width at 18 mA, 1.0 s, 100 pulse per second and 0.5 ms respectively to elicit hind limb tonic extension (HLTE). Thirty mice, 18 – 25 g were randomly divided into five groups (n=6). Group 1 was administered with distilled water (negative control, 10 ml/kg, *i.p.*). Groups 2 – 4 were treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg *i.p.* respectively of the EOLB while group 5 was administered with Sodium valproate 75 mg/kg *i.p.*. After 30 mins of pre-treatment with all test materials, each mouse was subjected to the MES test. Protection against HLTE was taken as a positive result. The abolition of hind limb tonic extension was considered as protection from electroshock (Potter *et al.*, 1984).

Influence of Flumazenil (2mg/kg; i.p.) on the extract of L. breviflora on novelty-induced behaviour (NIB)

The test was done to determine the effect of flumazenil (GABA_A receptor antagonist) on novelty-induced behaviour (locomotion) for the purpose of exploring the possible neurotransmitters or pathways through which the extract of *Lagenaria breviflora* exerts its effects and the probable mechanism of action. Six (6) groups of animals (n=6 per group) were employed in this experiment. Group 1 was administered distilled water (10 ml/kg), group 2 was treated with flumazenil (2 mg/kg, *i.p.*), group 3 was treated with 400 mg/kg of EOLB, *i.p.*, group 4 was treated with 400 mg/kg EOLB and flumazenil 2 mg/kg, *i.p.*, group 5 was treated with flumazenil 2 mg/kg, *i.p.* and diazepam 2 mg/kg, *i.p.* and group 6 was treated with diazepam 2 mg/kg *i.p.* Pre-treatment with the antagonist was 15 min prior to the administration of EOLB (Olayiwola *et al.*, 2013). The antagonist and EOLB were solubilized in distilled water. Each animal was placed in an observation cage after being injected with EOLB (400 mg/kg, *i.p.*), and observed for 20 min. (Oyemitan *et al.*, 2013).

Effect of the EOLB on amphetamine-induced hyperlocomotion in mice

The test was done to determine the effect of amphetamine on novelty-induced behaviour (locomotion) for the purpose of exploring the possible neurotransmitters or pathways through which the extract of *L. breviflora* exerts its effects and probable mechanism of action. Six (6) groups of animals (n=6 per group) were employed in this experiment. Group 1

was administered distilled water (10 ml/kg), group 2 was treated with amphetamine (2 mg/kg, *i.p.*), group 3 was treated with 400 mg/kg of EOLB, *i.p.*, group 4 was treated with 400 mg/kg EOLB and amphetamine 2 mg/kg, *i.p.*, group 5 was treated with amphetamine 2 mg/kg, *i.p.* and diazepam 2 mg/kg, *i.p.*, and group 6 was treated with diazepam 2 mg/kg *i.p.* Pre-treatment with the EOLB prior to the antagonist administration was 30 mins *i.p.* The antagonist and EOLB were solubilized in distilled water. Each animal was placed in an observation cage after being injected with EOLB (400 mg/kg, *i.p.*), and later amphetamine 2 mg/kg, *i.p.* and observed for 20 min. (Salahpour *et al.*, 2008).

Statistical analysis

The results obtained were expressed as the mean and standard error of the mean (Mean \pm SEM) and analyzed using a one-way analysis of variance (ANOVA), followed by Dunnett's or Student Newman-Keuls' test comparison between the treated groups and controls. The level of significance was set at a 95% confidence interval ($p < 0.05$) for all treatments carried out compared to control groups. Graph pad prism, version 5.01 (UK) was the statistical tool used.

RESULTS

Lethal dose (LD_{50}) estimation

The lethal doses (LD_{50}) values obtained were 1225 mg/kg and greater than 5000 mg/kg for the intraperitoneal and oral routes respectively.

Table 1: Acute toxicity profile of *Lagenaria breviflora* in mice

Dose mg/kg	Death pattern after 24 hours	
	Intraperitoneal (<i>i.p.</i>) route	Per oral (<i>p.o.</i>) route
	Phase 1 (n = 3)	
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
	Phase 2 (n = 1)	
1000	0/1	0/1
1500	1/1	0/1
2000	1/1	0/1
3000	1/1	0/1
4000	1/1	0/1
5000	1/1	0/1
LD_{50}	$\sqrt{(1000 \times 1500)} = 1224.75 \text{ mg/kg} > 5000 \text{ mg/kg (p.o.) (i.p.)}$	

The working doses chosen for the subsequent pharmacological studies were 100 mg/kg, 200 mg/kg and 400 mg/kg, *i.p.*

Effect of *Lagenaria breviflora* on novelty-induced behaviours (NIB)

The results from the novelty-induced rearing, grooming, and locomotion (number of squares crossed) showed that *L. breviflora* (EOLB) at 100, 200

and 400 mg/kg, *i.p.* and diazepam 2 mg/kg reduced rearing, grooming, and locomotion in mice significantly ($p < 0.01$) compared to the vehicle (Fig. 1A-C).

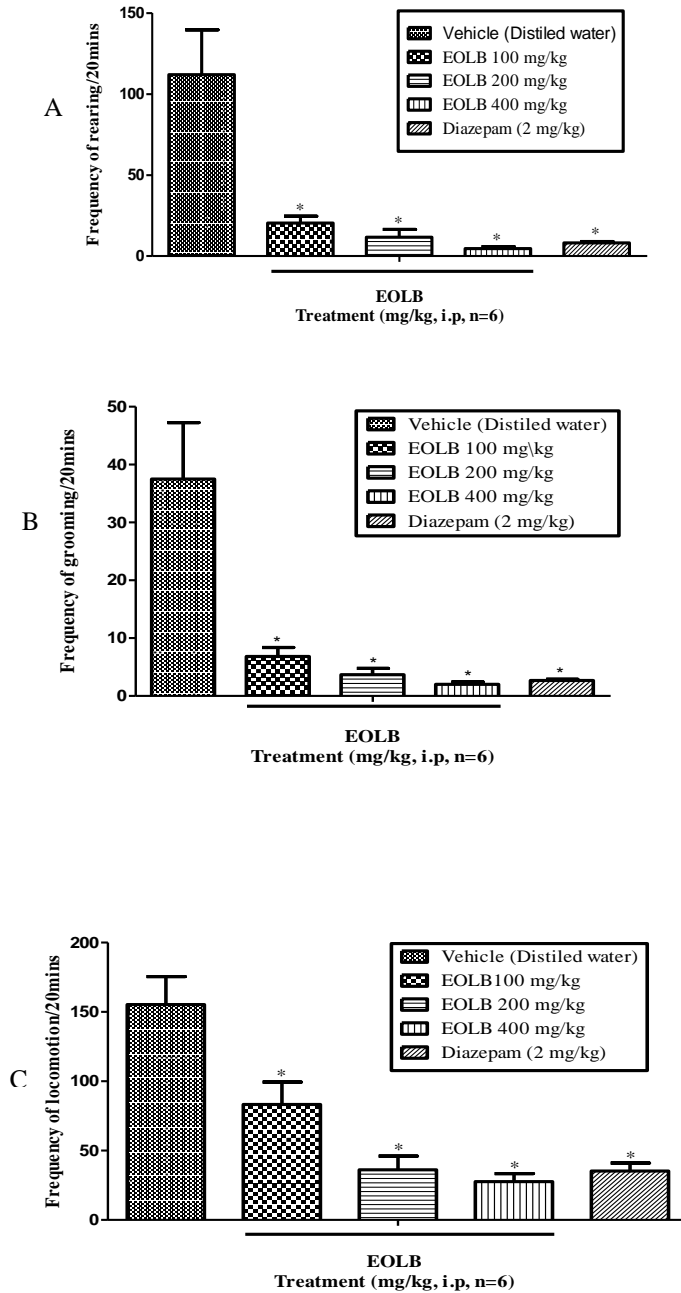


Figure 1: Effect of EOLB on novelty-induced rearing (A), grooming (B) and locomotion (C) in mice.

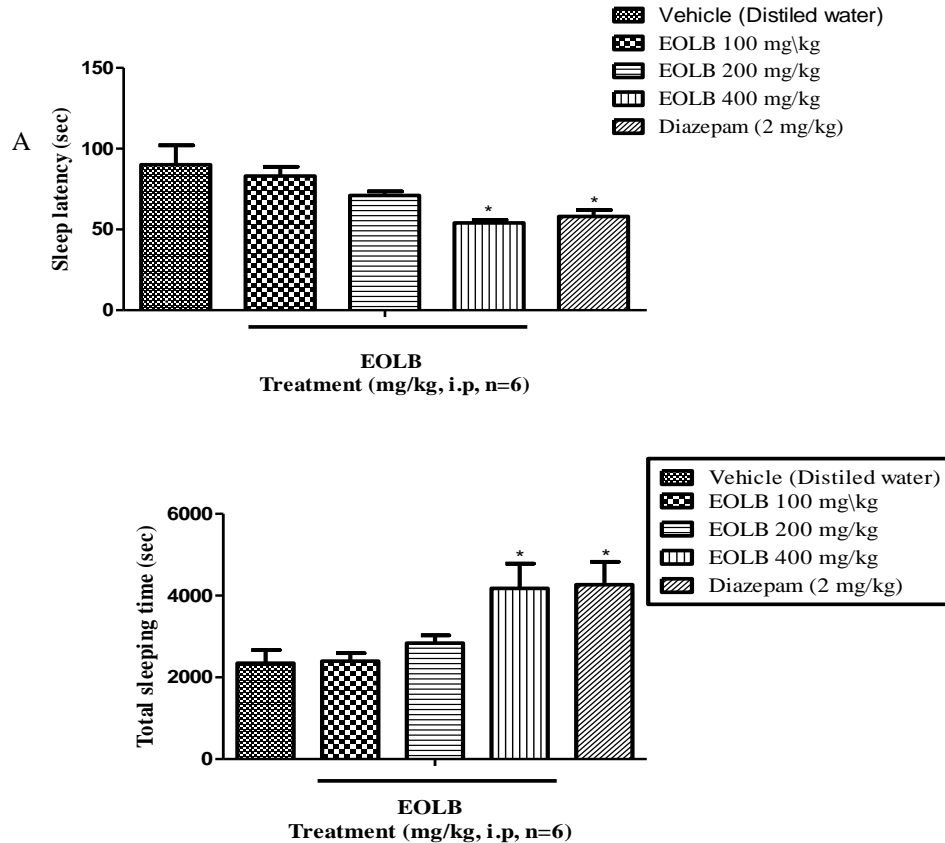
Each bar represents mean values with a standard error of mean \pm SEM, VEH, DZM and EOLB represent

vehicle (distilled water, 10 ml/kg, *i.p.*) diazepam (2 mg/kg *i.p.*) and extract of *Legenaria breviflora* respectively. * $p < 0.01$ statistically compared to vehicle (ANOVA, Dunnett's test).

Effects of the extract of *Legenaria breviflora* on ketamine-induced hypnosis

The extract of *L. breviflora* 400 mg/kg and diazepam 2 mg/kg, *i.p.* caused a significant [$p < 0.01$; $F_{(4, 25)} =$

5.9] reduction in the sleep latency, but a significant [$p < 0.05$; $F_{(4, 25)} = 5.3$] increase in the total sleeping time induced by Ketamine at 100 mg/kg *i.p.* when compared to the vehicle (distilled water, 10 ml/kg, *i.p.*) (Fig. 2A & B).



B

Figure 2: Effect of the extract of *Legenaria breviflora* on ketamine-induced sleep latency (A) and total sleeping time (B) in mice.

Each bar represents the mean value with a standard error of mean \pm SEM, and EOLB represents the extract of *Legenaria breviflora*. * = $p < 0.05$ statistically significant compared to vehicle (ANOVA, Dunnett's test).

Anticonvulsant test

Effect of the extract on maximal electro-shock (MES)-induced convulsion

The result obtained on the MES test showed that all the mice in the EOLB (100, 200 mg/kg and vehicle (distilled water, 10 ml/kg, *i.p.*) groups experienced

spontaneous hind limbs tonic extension (HLTE). The EOLB (400 mg/kg) and sodium valproate (75 mg/kg, *i.p.*) significantly ($p < 0.01$) shortened the recovery time in HLTE. However, 33.3% of the animals pretreated with extract 400 mg/kg and Sodium valproate 75 mg/kg *i.p.* completely blocked the HLTE in the MES test. There was no mortality in EOLB, vehicle and

sodium valproate treatment groups. The result is presented in Table 2.

Effect of the extract of *Lagenaria breviflora* pentylenetetrazole-induced convulsion

The EOLB (100 and 200 mg/kg) caused no significant increase in the convulsion latency (CL), compared to the vehicle (distilled water, 10 ml/kg, *i.p.*). EOLB (400 mg/kg) and diazepam (2 mg/kg), however, caused a significant ($p < 0.01$) increase in the time of death (TD) when compared to the vehicle. Furthermore, it was found that the EOLB 400 mg/kg offered 83.3% protection against PTZ-induced convulsion while diazepam (2 mg/kg) also offered 100% protection for the animals. The result is presented in Table 3.

Effect of the extract of *Lagenaria breviflora* on strychnine-induced convulsion test

Diazepam (2 mg/kg) caused a significant ($p < 0.01$) increase in both the convulsion latency and time of death compared to the vehicle. The EOLB 100, 200 and 400 mg/kg) showed no significant prolongation in both convulsion latency (CL) and time of death (TD) when compared to the vehicle. Neither diazepam (2 mg/kg) nor EOLB at all doses tested in this study offered protection against death. 100 % mortality was recorded in all the groups of animals. The result is presented in Table 4.

Table 2: Effect of Intraperitoneal administration of ethanol leaf extract of *Lagenaria breviflora* on hind limb extension (HLTE) in maximal electro-shock (MES) induced convulsion in mice.

Treatment	Ratios of MES-Induced HLTE	Duration of HLTE Mean \pm SEM (sec)	% Protection against HLTE	% Mortality
Vehicle 10 ml/kg	6/6	138 \pm 31.00	0.00	0.00
EOLB 100 mg/kg	6/6	129 \pm 11.00	0.00	0.00
EOLB 200 mg/kg	6/6	98 \pm 9.70	0.00	0.00
EOLB 400 mg/kg	2/6	124 \pm 6.90	33.33% ^{**}	0.00
Sodium valproate 75 mg/kg	2/6	93 \pm 25.00	33.33 % ^{**}	0.00

Data are expressed in mean \pm SEM of the ratio of MES-induced HLTE, duration of HLTE, percentage protection against HLTE and percentage mortality. (n = 6 per group). Significantly different from control: ANOVA followed by Dunnett’s test. Chi-square test for % mortality. ^{**} = $p < 0.01$.

Table 3: Effect of Intraperitoneal administration of ethanol leaf extract of *Lagenaria breviflora* on pentylenetetrazole (85 mg/kg *i.p.*) induced convulsion in mice

Treatment Groups <i>i.p</i> (n=6)	Convulsion Latency (CL) Mean \pm SEM (sec)	Time of Death Mean \pm SEM (TD) (sec)	Mortality %	Protection %
Vehicle 10 ml/kg	100.80 \pm 28.8	301.5 \pm 53.31	100%	0%
EOLB 100 mg/ kg	89.00 \pm 17.76	560.0 \pm 143.4	100%	0%
EOLB 200 mg/kg	115.50 \pm 37.65	480 \pm 266.5	100%	0%
EOLB 400 mg/kg	1147.00 \pm 292.100	1590 \pm 210.00 ^{**}	16.7% [*]	83.3% ^{**}
DZM 2 mg/kg	1550.00 \pm 250.00	1800.00 \pm 00 ^{**}	0% ^{**}	100% ^{**}

Data are expressed in mean \pm SEM of latencies to clonus and tonus, percentage mortality and percentage protection. (n = 6 per group). Significantly different from control: ANOVA followed by Dunnett’s test. Chi-square test for % mortality. ^{*} = $p < 0.05$ and ^{**} = $p < 0.01$.

Table 4: Effect of intraperitoneal administration of ethanol leaf extract of *Lagenaria breviflora* on strychnine-induced (4 mg/kg; i.p.) convulsion in mice

Treatment group(n=6)	Convulsion Latency (CL) MEAN ± SEM (sec)	Time of Death (TD) MEAN ± SEM (sec)	Mortality %	Protection %
Vehicle 10 ml/kg	144 ± 08.00	242 ± 27:00	100%	0%
EOLB 100mg/kg	104 ± 22.00	241 ± 27:00	100%	0%
EOLB 200 mg/kg	131 ± 13.00	200 ± 20:00	100%	0%
EOLB 400 mg/kg	121 ± 10:00	198 ± 30:00	100%	0%
Diazepam 5 mg/kg	206 ± 5:70**	552 ± 56.00**	100%	0%

Data are expressed in mean ± SEM of latencies to clonus and tonus, percentage mortality and percentage protection. (n=6 per group). Significantly different from control: ANOVA followed by Dunnett’s test. Chi-square test for % mortality. ** = p < 0.01.

Effect of ELOB on the influence of the antagonist (flumazenil, 2 mg/kg) on locomotion

Flumazenil caused a significant increase in locomotion compared to the vehicle, while EOLB 400 mg/kg alone caused a significant reduction in locomotion compared to the vehicle. The EOLB 400 mg/kg alone, and EOLB 400 mg/kg with flumazenil

significantly ($F_{(5, 30)} = 100$; p < 0.001) decreased locomotion in mice respectively compared to the vehicle (distilled H₂O). While flumazenil (GABA_A antagonist) also significantly reversed the inhibitory effect of the extract and diazepam by causing an increased frequency of locomotion higher than that caused by extract and diazepam. The result is presented in Figure 3.

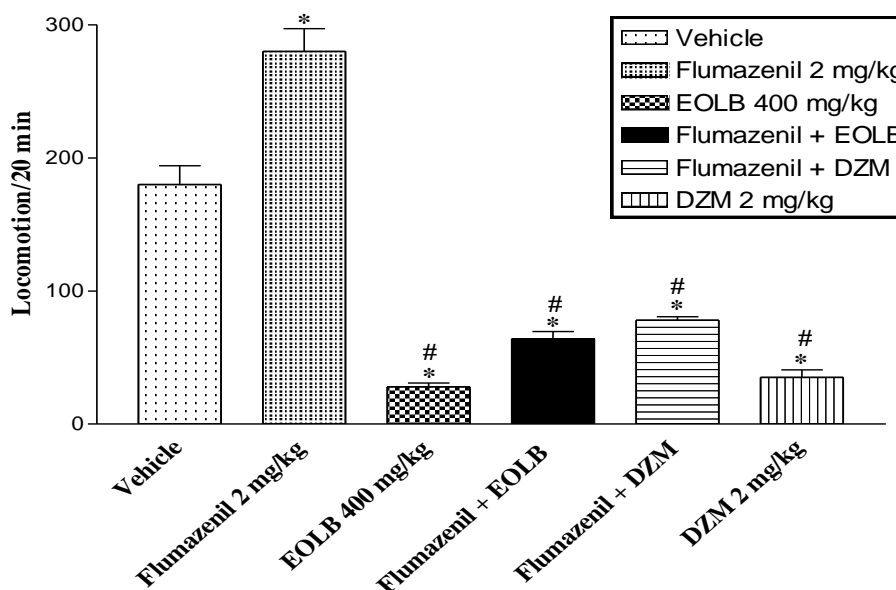


Figure 3: Influence of flumazenil on inhibition of locomotion caused by EOLB 400 mg/kg.

Each bar represents mean values with a standard error of mean ± SEM. DZM and EOLB represent Diazepam (2mg/kg) and extract of *L. breviflora* respectively. * = p < 0.001 statistically compared to vehicle and # = p < 0.001 to flumazenil (2 mg/kg) alone. (ANOVA, SNK).

Effect of the EOLB on amphetamine-induced hyperlocomotion

Amphetamine (2 mg/kg, *i.p.*) caused a significant increase ($F_{(5,30)} = 140$, $p < 0.001$) in the frequency of locomotion (a reversal of inhibition caused by the

EOLB). There was no statistically significant difference between the inhibition of locomotion caused by the EOLB and diazepam. The result is presented in Figure 4.

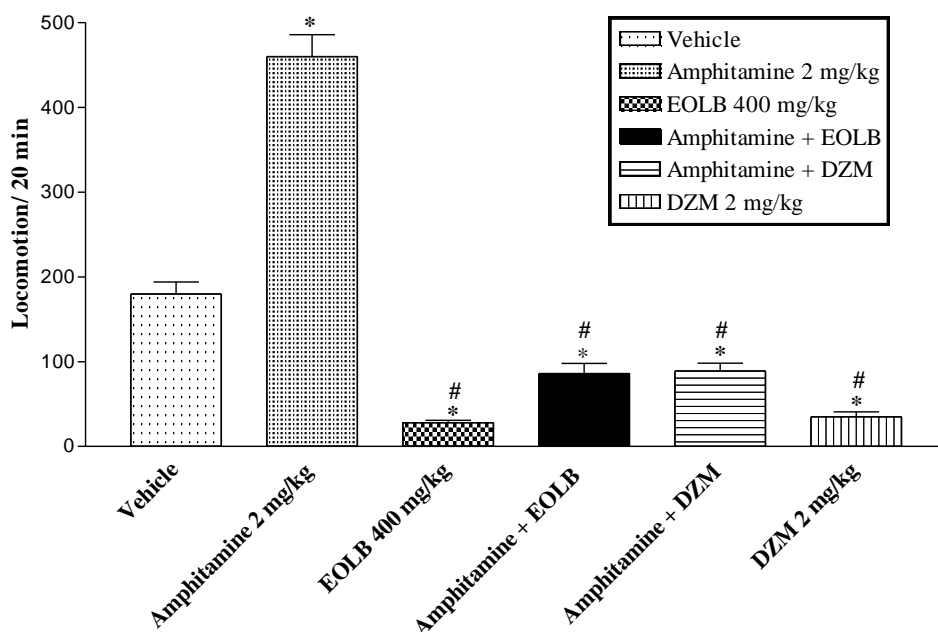


Figure 4: Effect of EOLB 400 mg/kg on hyperlocomotion induced by amphetamine (2 mg/kg) in mice.

Each bar is expressed as Mean ± SEM; (n = 6 per group). * = $p < 0.001$ compared to vehicle (distilled water, 10 ml/kg, *i.p.*), Diazepam (DZM) extract of *L. breviflora* (EOLB). # = $p < 0.001$ compared to the amphetamine (2 mg/kg) group. (ANOVA; SNK).

DISCUSSION

This study evaluated the acute toxicity profile, novelty-induced behaviour sedative and anticonvulsant effects of *Lagenaria breviflora* in mice.

The oral route LD_{50} was found to be greater than 5000 mg/kg as compared to 1224 mg/kg for the intraperitoneal route. The variation in the LD_{50} may be largely due to hepatic first-pass metabolism, enzymatic degradation, and other pharmacokinetic parameters (Okpako *et al.*, 2002). These features might have been responsible for the decreased concentration and bioavailability of the extract for the oral route compared to the intraperitoneal route. The oral route is perhaps the most preferred and widely used route for the administration of most medicinal preparations. However, the oral and intraperitoneal routes were used to investigate the acute toxicity profile of the extract *L. breviflora* (EOLB) and their relative toxicity profiles were determined.

The acute toxicity result for the two routes shows that the extract is non-toxic orally (Lorke, 1983), and could be harmless when used continuously which is peculiar to many traditional remedies, while for the intraperitoneal route, it was found to be mildly toxic at 1224 mg/kg (Lorke, 1983). However, it needs then to be stated that the extract would require more investigation using chronic or repeated dosing to further establish the toxicity potential of the extract both orally and intraperitoneally. The doses for this study were carefully chosen in geometric proportion, after consideration of the LD_{50} (1224 mg/kg) for the intraperitoneal route, hence 100 to 400 mg/kg were found to be suitable, since, this is still less than half of the LD_{50} value (1224 mg/kg).

The result of the novelty-induced rearing behaviour (NIB) showed that the extract of *Lagenaria breviflora* (EOLB) at 100, 200 and 400 mg/kg, *i.p.* and diazepam

2 mg/kg (positive control) reduced rearing in mice significantly ($p < 0.01$) compared to the vehicle. This is suggestive of CNS depression (Rang *et al.*, 2003). Rearing is part of the exploratory behaviour employed by rodents as one of the survival strategies in assessing a novel environment for food, sex, protection, and sometimes possibly escape. (Abdel-Barry and Al-Hakeim, 2000). The frequency of rearing and its modification in rodents can, therefore, be employed in evaluating test drugs and extracts for both sedative and central nervous system stimulation properties (Vogel, 2002). A high frequency of rearing behaviour indicates excitation while a low frequency indicates depression or an inhibitory effect (Oyemitan *et al.*, 2016). The result obtained in this study corroborates the depression of the extract in mice.

The effects of the EOLB (100, 200 and 400 mg/kg) and diazepam 2 mg/kg *i.p.* on grooming resulted in a significant ($p < 0.01$) reduction in grooming behaviour compared to the vehicle (distilled water, 10 mg/kg, *i.p.*). Grooming behaviour is related to fear or anxiety in rodents and it is an indication of behavioural adaptation to a stressful condition (Shaw *et al.*, 2007). Exposure of rodents to a new environment is alleged to be a stressful experience known to induce grooming behaviour and central dopaminergic activation has been reported in grooming behaviour via the D receptor (Scalzitti *et al.*, 1999).

The extract of *L. breviflora* at 100, 200, 400 mg/kg *i.p.* and diazepam 2 mg/kg (positive control), caused a significant ($p < 0.01$) reduction in the number of the lines crossed by the mice compared to the vehicle (distilled water, 10 mg/kg, *i.p.*). Locomotion is mediated mainly through the dopaminergic pathway while other neurochemical pathways have also been reported to modulate locomotive activities in animals (Rang *et al.*, 2003). A high frequency of this behaviour indicates excitation, while a low frequency indicates depression or an inhibitory effect on the CNS. The current result supports the inhibitory effects of the extract of *L. breviflora*, of the CNS (Nutt and Malizia, 2001).

Sedatives are known for their inhibitory effect on the CNS which is caused by either enhancement of GABA inhibitory effect by binding to GABA_A receptor-like benzodiazepines or antagonizing the effect of glutamate by inhibiting glutamate receptors such as N-methyl-D-aspartate (NMDA), AMPA, Kainite, glycine or metabotropic receptors (Rang *et al.*, 2007). Most of these substances have demonstrated a graded dose-dependent depression of the central nervous system function. At various graded doses, sedatives exhibit different pharmacological effects (i.e. hypnosis, general anaesthetic) which can be corroborated with the edge of central nervous system depression (Trevor & Way, 2009). Besides, ketamine

has been reported to cause sedation by additional GABA_A reporter potentiation (Karl *et al.*, 2008). The extra of *L. breviflora*, 400 mg/kg and diazepam *i.p.* cause a significant $p < 0.01$ reduction in the sleep latency by ketamine, 100 mg/kg, *i.p.* compared to the vehicle. The extract of *L. breviflora* (400 mg/kg *i.p.*) and diazepam (2 mg/kg) caused a significant ($p < 0.01$) increase in the total sleeping time induced by ketamine (100 mg/kg, *i.p.*). The decrease in SL and prolongation of TST are standard indications of sedation and sedative in this study activity of the extract of *L. breviflora*, further confirming the CNS inhibitory effect as earlier suggested in the NIB (Oyemitan *et al.*, 2016).

Substances that protect against the tonic-clonic seizures induced by PTZ are considered to be very useful to control myoclonic and absence seizures in humans (Carmody and Brennah, 2010). The effect of the extract of *L. breviflora* 400 mg/kg, *i.p.* on the PTZ-induced convulsion showed a significant ($p < 0.01$) increase in convulsion latency compared to vehicle (distilled water, 10 mg/kg, *i.p.*). Also, diazepam (2 mg/kg, *i.p.*) causes a significant ($p < 0.01$) increase in CL compared to the vehicle. The EOLB at 400 mg/kg and diazepam (2 mg/kg) significantly ($p < 0.01$) increased the time of death in mice compared to the vehicle. It was found that the extract of *L. breviflora* 400 mg/kg offered 83.3% protection against PTZ-induced convulsion respectively. Diazepam (2 mg/kg) also offered 100% protection against PTZ-induced mortality which also confirms earlier reports (Akula *et al.*, 2009). This prolongation of the time of death offered by EOLB is suggestive of anticonvulsant activity, possibly acting through the potentiation of the GABA pathway.

Clinically, this prolonged time of death offered by EOLB could be harnessed for other medical interventions to assuage the feats (Ayeh *et al.*, 2012). Strychnine acts by directly antagonizing glycine at the spinal cord and brain stem, thereby increasing the spinal reflexes (Oyemitan *et al.*, 2016). Agents that prolonged the onset of convulsion and death latency in the strychnine-induced convulsion model are said to possess antiepileptic capability (Adeyemi *et al.*, 2014). Diazepam caused a significant ($p < 0.01$) increase in the convulsion latency (CL) and an increase in the time of death (TD) compared to the vehicle (distilled water, 10 mg/kg, *i.p.*).

Furthermore, it was found that extract of *L. breviflora* at 100, 200 and 400 mg/kg offered 0% protection for all the mice. Diazepam (2 mg/kg) also offered no protection for the animals.

The extract at all doses tested in this present study gave a varying degree of protection against the maximum electric shock (MES) induced hind limb tonic extension (HLTE). The maximal electroshock (MES)

is a known tool to screen drugs for generalized tonic-clonic seizures. The MES disrupts signals or impulse transduction in the neurons due to the facilitation of Ca^{2+} influx into the cell, thus resulting in the prolongation of convulsion episodes (Inan and Buyukafsar, 2008). Besides modulating Ca^{2+} ion influx, MES is also proposed to facilitate the inflow of another positive ion such as Na^+ , which is blocked by a chemical agent and can prevent MES-induced hind limb tonic extension (Rang *et al.*, 2007). Phenytoin and sodium valproate are the main anticonvulsant drugs which utilize this mechanism of action (Rang *et al.*, 2007). The results from the MES model showed that the mice in the vehicle group demonstrated spontaneous hind limb tonic extension (HLTE) induced by the maximal electroshock (MES).

The EOLB (400 mg/kg) shortened the recovery time in HLTE. The EOLB at 400 mg/kg provided a 33.33% blockade against HLTE induced by MES. Similarly, Sodium valproate 75 mg/kg (positive control) also provided 33.33% protection against HLTE in this model. The EOLB in this study provided varying degrees of protection against HLTE, suggesting an anticonvulsant effect. The EOLB may be exhibiting its anticonvulsant activity through the blockade of Ca^{2+} influx similar to standard drugs such as Phenytoin and Sodium valproate (Rang *et al.*, 2003). It is worthy of note that these results can be used to support the ethnomedicinal use of this plant (NTMPA, 2012). Protection against HLTE caused by MES suggests the anti-convulsion potential of antiepileptics that prevent the spread of epileptic seizures from an epileptic focus during the seizure activities (Gupta *et al.*, 2012).

CONCLUSION

The results obtained in this research clearly indicated that the extract of *L. breviflora* demonstrated significant central nervous system inhibitory activities as seen in its ability to cause a significant reduction in rearing, grooming and locomotion. The extract of EOLB also showed significant sedative activity in the ketamine-induced hypnosis model, and anticonvulsant

An attempt was made to investigate the possible mechanism of the inhibitory action of the EOLB using the effect of an antagonist (flumazenil, 2 mg/kg, *i.p.*) and an agonist (amphetamine, 2 mg/kg, *i.p.*) on the activity of the EOLB on novelty induced locomotion (NIL) in the open-field model. The pre-treatment with flumazenil prior to the administration of the extract reversed the inhibitory effect of EOLB on locomotion significantly ($p < 0.001$) This result buttressed the inhibitory effect of the extract (on rearing, grooming and locomotion), and thus suggestive of the extract working through the augmentation of GABAergic neurotransmission (Nutt and Malizia, 2001). Since flumazenil (GABA_A receptor antagonist) was able to reverse the effect partially, there could be other mechanisms involved since the effect of the extract on NIL was not a complete reversal. GABA and GABA_A receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours (Nutt and Malizia, 2001).

Locomotion is mediated mainly through the dopaminergic pathway (Rang *et al.*, 1999). Amphetamine (a Dopamine agonist) which significantly potentiates dopaminergic transmission and the attendant hyper-locomotor response was found in this study to reverse the effects of the extract on locomotion behaviour, thus suggesting the mediation of the inhibitory effect through dopaminergic neurotransmission, which therefore supports its sedative activity in mice (Rang *et al.*, 2007).

effect in the PTZ, STR, and MES-induced convulsion models. The mechanism of action of the extract of *Lagenaria breviflora* could be through the augmentation of GABAergic neurotransmission and or inhibition of the dopaminergic neurotransmission or could be by other mechanisms.

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*Address for correspondence: Joshua Oloruntobi Imoru
Department of Pharmacology,
Faculty of Pharmacy,
Obafemi Awolowo University,
Ile-Ife, Nigeria.
Telephone: +2348035149838
E-mails: tobiimoru@yahoo.com

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