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Behavioural activities and chemical composition of fresh leaf essential oil of *Plectranthus aegyptiacus* from Southwest Nigeria in mice

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Abstract

Objectives: This study determined the chemical composition of essential oil obtained from fresh leaf of *Plectranthus aegyptiacus*, and evaluated it for novelty-induced behavioural (NIB) and determine its mechanism(s) of action in mice.

Methods: The oil was hydro-distillated, and analyzed using gas chromatography-mass spectrometry. The effects of the oil (50, 100 and 150 mg/kg; i.p., n=6) on novelty-induced behavioural was assessed using open field test and head dipping on hole board. Probable mechanism(s) were evaluated using antagonists: flumazenil, naloxone and cyproheptadine at 2 mg/kg each, atropine and yohimbine at 5 mg/kg and 1 mg/kg respectively.

Key Findings: The LD50 values obtained were 2154 and 490 mg/kg for oral and intraperitoneal routes respectively. The oil (50, 100 and150 mg/kg) significantly ($p<0.05$, 0.01 and 0.01) inhibited all NIB and head dips. Flumazenil significantly ($p<0.05$) reversed the effect of the oil on NIB; atropine, naloxone and cyproheptadine significantly ($p<0.01$, 0.01 and 0.001) potentiated inhibitory effect on NIB respectively, while yohimbine showed no significantly effect. The analyzed oil showed 61 compounds, and the major compounds were carvacrol, germacrene-D, p-cymene and [1,1'-Bicyclopentyl]-2,2'-diol.

Conclusions: The study concluded that the oil possessed central nervous system depressant activity, which could be mediated mainly through augmentation of GABAergic neurotransmission, while cholinergic-(muscarinic), adrenergic and serotonergic pathways may be involved.

Keywords: Acute toxicity; Carvacrol; Essential oil; Novelty-induced behavior; *Plectranthus aegyptiacus*

1. Introduction

The use of plants for treating ailments and diseases is as old as human species, although it is difficult to point to an exact time. The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh) which are still used today to treat coughs, colds and inflammation [1]. *Plectranthus* is one of the largest genera of family Lamiaceae, which belongs to subfamily Nepetoideae, tribe Ocimeae and subtribe Plectranthinae, containing about 300 species found in warm and tropical regions of the world [2], including Africa, Asia and Australia, and of the 300 species of *Plectranthus*,

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62 species are reported to be used as medicines, ornamentals, foods, flavours and fodder [3]. *Plectranthus aegyptiacus,* synonym *P. tenuiflorus* (Vatke) Agnew, differing in their essential oil compositions [4] is widely spread in tropical Africa, including Nigeria, where it is commonly known as *Efinrin-Oyinbo* and *Kpo-Oyibo* among the Yoruba and Epie speaking people of southern Nigeria respectively.

In folkloric medicine, *P. aegyptiacus* is used for treating pain, sensory diseases [3, 5] ear ache, sore throat, respiratory system infections, and abdominal disorders [6]. Several studies have identified diverse chemical compounds in the essential oil of *Plectranthus aegyptiacus* and notable ones include myrcene, limonene, camphor, borneol, terpinen-4-ol, α-terpineol, α-cubebene, β-cubebene, caryophyllene oxide, β-caryophyllene among others [7-9], also reported are octen-3-ol, α-terpinene, terpinene-4-ol, γ-terpinene, α-terpinyl acetate, linalool, carvacrol, β-bourbonene, β-elemene, α-gurjunene, γ-elemene, aromadendrene, germacrene D, γ-muurolene and α-amorphene among others as constituents of the plant leaf [10]. Recently, we evaluated the essential oil of the fresh leaf of this species in our laboratory and results obtained showed that it demonstrated significant sedative, anticonvulsant and analgesic activities in mice [11]. However, the chemical composition of the oil used was not analysed hence, this study aimed at evaluating the oil for behavioural activities, determine its mechanism(s) of action in mice as well as characterizing its chemical constituents

2. Material and methods

2.1. Plant Collection and Identification

The fresh leaves of *Plectranthus aegyptiacus* were collected between July and September 2016, at the Medicinal Garden, Faculty of Pharmacy, on the campus of Obafemi Awolowo University, Ile-Ife, Southwest,Nigeria. The plant was initially identified and authenticated by Mr. I. I. Ogunlowo, Department of Pharmacognosy Faculty of Pharmacy, Obafemi Awolowo University, and further identified by Mr. G. A. Ademoriyo, Botany Department, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria, and herbarium specimen number IFE – **17624** was issued.

2.2. Equipment and Reagents

Weighing balance (Mettler Toledo), animal cages, plexiglas cage, electroconvulsiometer, hole board, Clevenger-type distillatory apparatus.

2.3. Drugs

Diazepam (Valium®, Roche, Switzerland), atropine (Vixa Pharmaceutical Co. Ltd., China), cyproheptadine (Therapeutic laboratories Nig. Ltd., Lagos, Nigeria); yohimbine, naloxone and flumazenil (Sigma),

2.4. Experimental Animals

Adult male mice (18-25 g) obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria were used for the study.

2.5. Ethical approval

The animal experiments were carried out according to the protocol approved by the committee on animal use and care of the Obafemi Awolowo University, Ile-Ife, Nigeria.

3. Methods

3.1. Extraction of essential oil

The volatile fraction of *P. aegyptiacus* fresh leaf was obtained by hydrodistillation for about 4 h using Clevenger-like apparatus, at the Postgraduate Toxicology Laboratory, Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile – Ife, Nigeria.

3.1.1. Distillation Procedure

400 g of the fresh leaves of *P. aegyptiacus* was weighed, washed and packed into the round bottom flask component of the Clevenger-like apparatus and two litres of distil water was measured and added into the flask containing the fresh leaves of the plant and each distillation process took 4 h. The flask was then mounted on the electrically powered heating mantle component of the Clevenger apparatus. Distilled oil was collected, dried on sodium sulfate crystals and stored

in amber bottle at 4°C. The yield of the oil varies between 0.05 and 0.20 % w/w, with a determined and estimated density of 1.03 g/ml.

3.2. Chemical analysis of the essential oil

The chemical composition of the essential oil of *P. aegyptiacus* was determined using

Gas Chromatography-Mass Spectrometry (GC/MS) analysis at the Central Analytical Facilities, Stellenbosch University, Stellenbosch, South Africa. GC analysis was carried out on a Perkin-Elmer 8500 gas chromatograph with a FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25µm). The operating conditions were as follows: carrier gas, helium with a flow rate ranging from 6 mL/min to 10mL/min; column temperature ranged from 50 °C to 280 °C at 5 °C/min; injector and detector temperature were at 280°C; volume injected at 0,1µl of the oil; split ratio. 1:50. The GC/MS analysis was performed on a Hewlett Packard 6890 MS selective detector coupled with Hewlett Packard 6890 gas chromatograph equipped with a cross-linked 5% PHME siloxane HP-5MS capillary column (30 m x 0,25 mm; film thickness 0,25 µm) and operating is the same conditions as described above. The MS operating parameters were as follows: ionization potential 70 eV, ionization current 2A, ion source temperature 200°C, and resolution 1000. The percentage of the samples was computed from the GC peak areas without using correction for response factors.

3.3. General experimental design

Animals were randomly selected into 5 groups (n=6). Group I serve as the negative control and received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with the oil at doses of 50, 100 and 150 mg/kg respectively, while the positive control group received the appropriate standard drug.

3.4. Determination of LD_{50}

The method described by Lorke, [12] was used to determine the LD_{50} values. This model involves two phases. In the first phase, three increasing doses of 10, 100 and 1000 mg/kg of the oil 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 $LD_{50} = \sqrt{A \times B}$

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

3.5. Effect of the essential oil of *P. aegyptiacus* on novelty-induced behaviour

Open field test (OFT) was adopted [13], with minor modification [14] was used to test novelty-induced behavioural activities of rearing (number of times the animal stand on its hind-limbs with fore limbs in the air or against the wall), grooming (nose, face and mouth washing) and locomotion (number of squares crossed with all limbs) in mice. The male mice were randomly divided into six groups (n=6 per group). Group I received 5% Tween – 80 (10 ml/kg or 0.1 ml/10 g) as control intraperitoneally (i.p.), Group II, III and IV were administered with different doses (50, 100 and 150 mg/kg) of essential oil of *P. aegyptiacus* (EOPA) respectively via intraperitoneal (i.p.) route and group V diazepam (2.5 mg/kg, i.p.) to serve as the positive control. All the mice were pre-treated (30 min) prior to assessment for rearing, grooming and locomotion for 20 min.

The mice were placed directly from the home cages into an opaque plexiglass observation cage (45 x 25 x 25 cm) with only one side transparent for observation. All mice were observed and assessed singly, used only once, and the Plexiglas cleaned with 70% alcohol after each assessment to remove olfactory cue from one animal to the other [15].

3.6. Effects of the essential oil of *Plectranthus aegyptiacus* on head dips

The hole board is a flat space of 25 cm^2, with 16 holes (each 3 cm in diameter). After 30 min pretreatment, each mouse was placed gently at the centre of the hole board from the home cages. The number of head–poking (head dips) demonstrated by each mouse was counted and recorded for 5 min. [14].

3.7. Influence of some antagonists on the effect of essential oil of *P. aegyptiacus* on novelty-induced behaviour (NIB)

The test was carried out to determine the influence of some antagonists effect of the essential oil of *P. aegyptiacus* on novelty-induced behaviour. Sixty six mice were randomly allocated into eleven groups (n=6). Group I was administered with 0.1 ml vehicle, while groups II-XI were treated with atropine (muscarinic receptor antagonist, 0.5 mg/kg, *i.p.*), atropine (0.5 mg/kg) plus EOPA (150 mg/kg); cyproheptadine (5-HT_2 receptor antagonist, 2 mg/kg, *i.p*), cyproheptadine (2 mg/kg) with EOPA (150 mg/kg); yohimbine (α2- adrenergic receptor antagonist, 1.0 mg/kg, *i.p.*), yohimbine (1.0 mg/kg) with EOPA (150 mg/kg); flumazenil ($GABA_A$ receptor antagonist, 2.0 mg/kg, *i.p.*), flumazenil (2.0 mg/kg) with EOPA (150 mg/kg); naloxone (μ opioid receptor antagonist, 2 mg/kg *i.p.*) and naloxone (2.0 mg/kg) with EOPA 150 mg/kg respectively. Pretreatment with the antagonists prior to the oil was 30 min, except flumazenil and naloxone which was pretreated 15 min [16]. Each animal was placed singly inside the plexiglas cage after injected with EOPA (150 mg/ kg *i.p.*), and observed for the number of locomotion, rearing and grooming for 20 min [16-17].

3.8. Statistical Analysis

The results were expressed as Mean ± SEM, and analyzed using ANOVA, followed by Dunnett's comparison. The level of significance was set at 95% confidence interval at $p<0.05$ for all treatment. The results of the influence of antagonists on the effect of essential oil were analyzed with ANOVA, followed by Student–Newman-Keuls post hoc test. Graph pad prism, version 5.01 (UK) was used

4. Results and discussion

4.1. Chemical composition of the essential oil of *P. aegyptiacus* fresh leaf

The results showed that 61 compounds were detected, 52 were fully identified. The major compounds (≥ 3.55%) were Carvacrol (8.45%), germacrene-D (8.32%), p-cymene (7.20%), 1,1'-bicyclopentyl -2,2'-diol (5.97%), δ-cadinene (5.0%), caryophyllene oxide (4.98%), (-)-humulene epoxide II (3.75%), α-copaene (3.73%) and α-humulene (3.55%). Some of these compounds are shown in Figure 1.

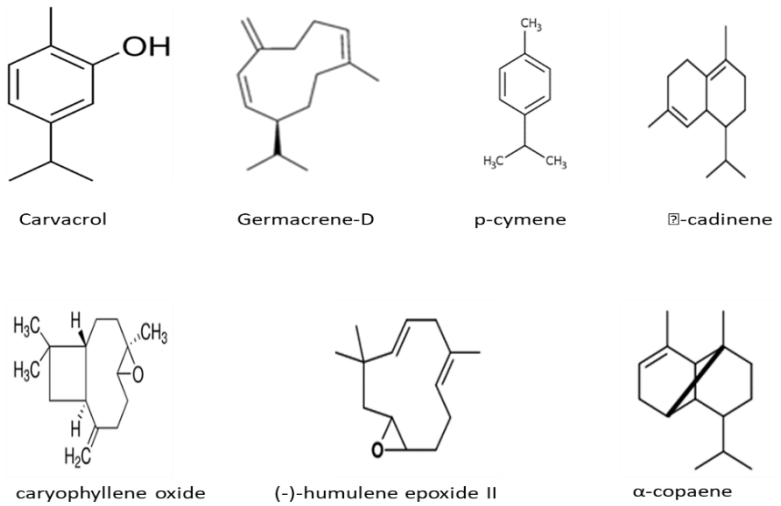

Figure 1 Chemical structures of some compounds identified in the essential oil of *P. aegyptiacus*

Table 1 Chemical composition of the essential oil *P. aegyptiacus* fresh leaf

Peak number	Library/ ID	RT/Sec	KI value	CAS-Number	%
1	α-pipene	5.432	934	000080-56-8	0.24
2	1-Octen-3-ol	6.817	977	053907-72-5	0.53
3	α-Terpipene	7.475	1018	000099-86-5	2.82
4	p-Cymene	7.876	1026	000099-87-6	7.20

5	Trans-β-Ocimene	8.160	1050	003779-61-1	1.35
6	ɣ-terpinene	8.529	1059	000099-85-4	2.23
7	Linalool l	9.7217	1101	000078-70-6	1.12
8	Terpinen-4-ol	11.883	1177	000562-74-3	0.75
9	Carvacrol	15.086	1285	000499-75-2	8.45
10	Dimethylhexynediol	15.177	1323	000142-30-3	0.19
11	α-Cubebene	15.226	1345	017699-14-8	0.27
12	α-Copaene	16.017	1376	003856-25-5	3.73
13	β-Cubebene	16.279	1389	013744-15-5	0.29
14	β-Elemene	16.397	1391	000515-13-9	0.71
15	α-Gurjunene	16.670	1409	000489-40-7	0.44
16	β-Caryophyllene	17.098	1418	000087-44-5	1.62
17	Unknown	17.285	-----	013744-15-5	0.37
18	α-Humulene	17.985	1440	006753-98-6	3.55
19	α-amorphene	18.365	1453	023515-88-0	0.38
20	Germacrene-d	18.606	1480	023986-74-5	8.32
21	β-Selinene	18.734	1485	017066-67-0	0.40
22	α-Muurolene	18.841	1499	031983-22-9	1.08
23	Unknown	18.954	-----	111005-47-1	1.09
24	δ-Cadinene	19.269	1504	000483-76-1	5.00
25	Methyl 3,5 bis(ethylamino)benzoate	19.472	1510	000000-00-0	1.92
26	1S,cis-Calamenene	19.537	1517	000483-77-2	2.25
27	Elema-1,3-dien-6.alpha.-ol	19.638	1521	035727-45-8	1.66
28	trans-1-Chloro-1,2dimethylsilacyclohexane	20.275	1538	000000-00-0	0.64
29	1,6-Germacradien-5-ol	20.836	1547	000000-00-0	0.91
30	(-)-Caryophyllene oxide	21.002	1568	001139-30-6	2.36
31	Caryophyllene oxide	21.077	1573	001139-30-6	4.98
32	Viridiflorol	21.216	1590	000552-02-3	0.32
33	d-Ledol	21.393	1596	000577-27-5	0.32
34	(-)-Humulene epoxide II	21.660	1606	019888-34-7	3.75
35	Unknown	21.842	------	016728-99-7	0.38
36	Copaene	22.082	1614	003856-25-5	0.62
37	.alpha.-cadinol	22.147	1627	000481-34-5	1.15
38	(.+-.)-Torreyol	22.248	1641	071300-63-5	1.69
39	T-Muurolol	22.516	1645	019912-62-0	2.62
40	Unknown	22.580	-----	000483-77-2	0.79
41	Unknown	22.981	-----	081571-58-6	1.31
42	Calamenene	23.190	1726	000483-77-2	0.70

43	[1,1'-Bicyclopentyl]-2,2'-diol	23.393	1732	073522-20-0	5.97
44	2-Furyl-cyclohexyl-1,4-dioxide	23.425	1750	056666-97-8	0.23
45	1,4-Diisopropyl-2-methylbenzene	23.778	1755	058502-85-5	1.95
46	2-Thiopyridone	24.136	1764	002637-34-5	0.53
47	(2R,5E)-caryophyll-5-en-12-al	24.345	1798	000000-00-0	0.68
48	Calamenene	24.452	1811	000483-77-2	1.60
49	1S,Cis-Calamenene	24.773	1836	000483-77-2	0.19
50	Unknown	24.896	-----	087422-06-8	1.73
51	Unknown	25.730	-----	097549-16-1	2.26
52	Unknown	25.827	-----	075750-99-1	1.07
53	1-deoxycapsidiol	25.944	1843	000000-00-0	0.59
54	(Z)-valerenyl acetate	26.137	1862	000000-00-0	0.32
55	retro-Ionone	26.533	1884	056052-61-0	0.45
56	Unknown	26.757	-----	055683-15-3	0.69
57	Unknown	26.870	-----	063780-12-1	0.35
58	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)	27.046	1908	062337-99-9	0.24
59	α-Farnesene	27.137	1920	000502-61-4	0.36
60	1-chloro-2,4-dimethoxy-3-methylphenol	27.426	1986	105104-00-5	0.15
61	Phytol	28.426	2111	000150-86-7	0.15

4.2. Effect of the essential oil of *P. aegyptiacus* on novelty-induced behaviours (NIB)

4.2.1. Effects of the essential oil on novelty-induced rearing in mice

The results from the novelty- induced rearing showed that essential oil of *Plectranthus aegyptiacus* (EOPA) at 50, 100 and 150 mg/kg, i.p. and diazepam 2.5 mg/kg reduced rearing in mice significantly [$p< 0.01$; $F_{(4, 29)} = 10.036$] compared to the vehicle (Fig. 2A).

4.2.2. Effects of the essential oil on novelty-induced grooming in mice

The essential oil of *Plectranthus aegyptiacus* (EOPA) at 50, 100 and 150 mg/kg, i.p., and the positive control (diazepam 2.5 mg/kg) reduced grooming in mice significantly [$p< 0.01$; $F_{(4, 29)} = 17.945$] compared to the vehicle (Fig. 2B).

4.2.3. Effects of the essential oil on novelty induced locomotion in mice

The oil at all doses (50, 100 and 150 mg/kg, i.p.) and diazepam (2.5 mg/kg, i.p.) caused a significant [$p<0.05$-0.01; $F_{(4, 29)} = 7.003$] reduction in the number of square crossed compared to the vehicle (Fig. 2C)

4.3. Effects of the essential oil on head dip

The oil (50 mg/kg) caused insignificant reduction in head dips compared to the vehicle (5% Tween-80). While the oil at (100 and 150 mg/kg) and diazepam (2.5 mg/kg) caused a significant [$p>0.01$; $F_{(4, 29)} = 11.076$] decrease in head dips compared to the vehicle (Fig. 3).

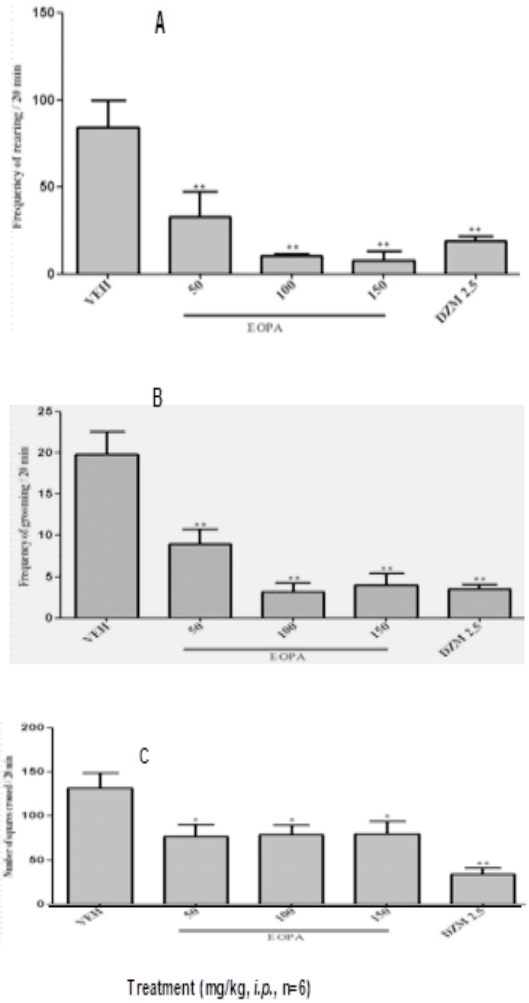

Figure 2 Effect of EOPA on novelty- induced rearing (A), grooming (B) and locomotion (C) in mice

Bars represent mean values with standard error of mean±SEM, (n=6). VEH, DZM and EOPA represent vehicle (5% Tween 80), diazepam and essential oil of *Plectranthus aegyptiacus* respectively.

**p< 0.05 – 0.01, statistically significant compared to vehicle (ANOVA, Dunnett's test)

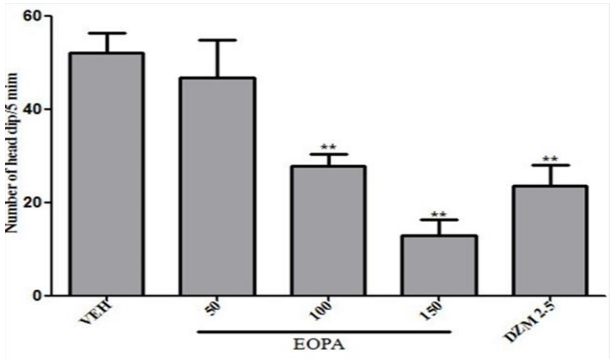

Figure 3 Effect of EOPA on head dipping in mice

Bars represent mean values with standard error of mean±SEM, (n=6) VEH, DZM and EOPA represent vehicle (5% Tween 80), diazepam and essential oil of *Plectranthus aegyptiacus* respectively.

**p< 0.01 statistically significant compared to vehicle (ANOVA, Dunnett's test)

4.4. Influence of the antagonists on the effect of oil on rearing, grooming and locomotion in mice

4.4.1. Influence of the antagonists on the effect of the oil on rearing

Essential oil of *Plectranthus aegyptiacus* (EOPA) alone, and the EOPA with atropine, yohimbine naloxone and cyproheptadine significantly [$p<0.01$- 0.001; $F_{(11, 60)} = 6.601$] decreased rearing behaviour in mice respectively compared to the vehicle (5% Tween -80), while flumazenil (GABAA antagonist) reversed the effect of the oil (Fig. 4A).

4.4.2. Influence of the antagonists on the effect of the oil on grooming

Results for the novelty - induced grooming, showed that the essential oil of *Plectranthus aegyptiacus*, atropine, naloxone, cyproheptadine and flumazenil alone significantly [$p<0.05$ – 0.001; $F_{(11, 60)} = 8.676$] decreased grooming behaviour in mice respectively compared to the vehicle (5% Tween – 80). While the oil plus atropine, yohimbine, flumazenil, naloxone and cyproheptadine respectively were observed to also significantly ($p<0.001$) reduced grooming behaviour in mice respectively compared to the vehicle (Fig. 4B).

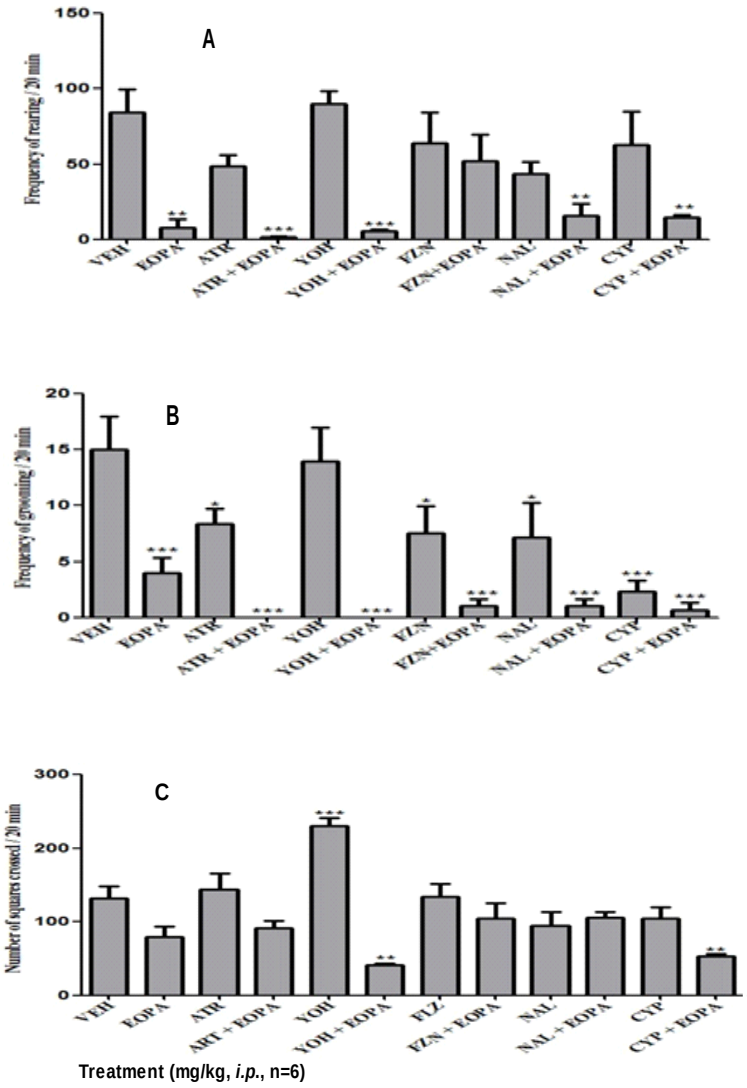

Figure 4 Influence of the antagonists on the effect of oil on rearing (A), grooming (B) and locomotion (C) in mice

Bars represent mean values with standard error of mean ±SEM. VEH, EOPA, ATR, YOH, FZN, NAL and CYP represent vehicle (5% Tween 80), essential oil of *Plectranthus aegyptiacus* (150 mg/kg), atropine (0.5 mg/kg), yohimbine (1 mg/kg), flumazenil (2 mg/kg), naloxone (2 mg/kg) and cyproheptadine (2 mg/kg) respectively.

*$p<0.05$ - 0.001, statistically significant compared to the vehicle (ANOVA, Student-Newman-Keuls test).

4.4.3. Influence of the antagonists on the effect of the oil on locomotion

The antagonists (yohimbine and cyproheptadine) plus EOPA (150 mg/kg) caused a significant [$p<0.01$; $F_{(11, 60)} = 11.374$] reduction in locomotion (square crossing) in mice compared to the vehicle respectively. While the other antagonists (naloxone, flumazenil and atropine) plus EOPA exhibited no significant decrease in locomotion compared to the vehicle. However, yohimbine alone exhibited a significant [$p<0.001$; $F_{(11, 60)} = 11.374$] increase in locomotion compared to the vehicle. Other antagonists (naloxone, flumazenil, cyproheptadine and atropine) alone caused on significant difference in locomotion compared to the vehicle (Fig. 4C).

5. Discussion

This study evaluated the effects of essential oil of *Plectranthus aegyptiacus* (EOPA) on novelty- induced behaviour (NIB), and its probable mechanism(s) on the various neural pathways in mice. Furthermore, the chemical composition of the oil of the plant fresh leaf was also determined. The results obtained from this research work showed generally, that the oil exhibited significant effect on the central nervous system.

Acute toxicity is the first step in unveiling the toxicity profile of unknown substances, including medicinal plant extract, isolate, natural products and related chemicals. The index of acute toxicity is the LD_{50}, which provide preliminary information about acute toxicity irrespective of the substance, dose ranges, as well as for all routes of administration [12]. The acute toxicity profile of the essential oil of *P. aegyptiacus* was investigated for both the oral and intraperitoneal routes, and their relative toxicity were determined. For the oral route, the LD_{50} was found to be 2154 mg/kg (p.o) compared to 490 mg/kg (i.p.) for the intraperitoneal route. The oral route is perhaps the most preferred and widely used ethnomedicinally for administration of most medicinal preparations. The variation in the LD_{50} of the oral route compared to the intraperitoneal route may be due to hepatic first pass metabolism, enzymatic degradation and other pharmacokinetic parameters [18]. These factors might have decreased the concentration and bioavailability of the oil for the oral route compared to the intraperitoneal.

The intraperitoneal route however, has been found to be faster and gave more consistent results that are readily reproducible [19], hence its choice in this study. The acute toxicity result for the two routes showed that the oil is slightly toxic orally (2154 mg/kg) (Lorke, 1983), and can be harmful when used chronically, which is peculiar to many traditional remedies. While for the intraperitoneal route, the oil was found to be moderately toxic (490 mg/kg) [12]. The implication of this result is that the oil may need further probing using sub-chronic and chronic toxicity models to further establish the toxicity potential of the oil both orally and intraperitoneally. The doses for this study were carefully chosen in multiples of 50 mg/kg (arithmetic progression), after consideration of the LD_{50} (490 mg/kg) for the intraperitoneal route, hence 50-150 mg/kg, *i.p*. were found to be suitable, since it is still less than half of the LD_{50} value (490 mg/kg) obtained.

The major effect of the oil was found to be depression of central nervous system, corroborating its potent sedative property which is consistent with studies on some essential oil [20]. The inhibitory effects of the oils on exploratory behaviour became manifested within 5 min after intraperitoneal administration of EOPA as observed during assessment. The novelty- induced behavioural responses are the results of the integration of all the processes ongoing in the underlying organ or system (especially the central nervous system) in interaction with the external and physical environment [21] Rodents display novelty - induced behavioural syndromes (rearing, grooming, scratching and wet dog shakes) on exposure to new environment[12]. The novelty-induced behavioural responses are known to be regulated by multiple neurotransmitter systems: gamma-aminobutyric acid (GABA), acetylcholine (Ach), dopamine, opioid, serotonin (5-HT), benzodiazepines and many others yet to be ascertained [17].

The novelty induced-rearing is regulated by multiple neurotransmitter systems [22], such transmitters include acetylcholine (ACh), dopamine, serotonin (5-HT), gamma-amino butyric acid (GABA), opioid and noradrenaline [22-24]. The result of the novelty-induced rearing behaviour showed that all doses of EOPA (50, 100 and 150 mg/kg, i.p.) used in this study significantly ($p<0.01$) reduced rearing in a dose-dependent manner in the mice compared to the vehicle. At 100 and 150 mg/kg, there was a significant ($p<0.01$) decrease in rearing compared to the control (diazepam 2.5 mg/kg, i.p.). Rearing is part of the exploratory behaviour employed by rodents [25]. Measurement of frequency of rearing in rodents can therefore be used in assessing test drugs and extracts for both sedative property and central nervous system stimulation [26]. The results obtained in this study confirmed the sedative activity of the oil in mice.

The effects of the EOPA on novelty-induced grooming behaviour showed significant ($p<0.01$) decrease at all doses (50, 100 and 150 mg/kg, i.p.) of the oil and diazepam 2.5 mg/kg, i.p., compared to the vehicle. Grooming patterning emerges as a sensitive index of altered animal anxiety and emotionality [27]. Gamma-aminobutyric acid (GABA) is the primary

mediator of inhibitory transmission in the mammalian central nervous system [28]. GABA and its $GABA_A$ receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours [29], including grooming [30]. At the same time, dysfunctions of the GABAergic system have long been associated with stress and anxiety spectrum disorders [29]. Depression of the grooming behaviour is therefore suggestive of a possible anxiolytic property of the EOPA [31].

The result of the EOPA (50, 100 and 150 mg/kg) on locomotion showed a significant ($p<0.05$) reduction compared to the vehicle. While diazepam (2.5 mg/kg, i.p.) also caused a significant ($p<0.01$) decrease in number of squares crossed compared to the vehicle. Locomotion is mediated mainly through dopaminergic pathway. A high frequency of this behaviour indicates excitation, while low frequency of this behaviour indicates depression or inhibitory effect. However, other neurochemical pathways have been reported to modulate locomotive activities in animals. These results further confirmed the inhibitory effect of the oil on locomotion, which is a common property of CNS depressant. Considering the effect of the oil on NIB, it can be suggested that the major action of the oil is depression of the central nervous system [26, 29].

Conspicuously, the general effect of the essential oil *P. aegyptiacus* in the mice is CNS depression. Consequently, following the confirmation of the inhibitory effect exhibited by the oil on novelty-induced behaviours (grooming, rearing and locomotion) and head dipping in this study, it became imperative to explore the mechanism of action of the oil using pharmacological antagonism of the various neurochemical pathways.

Atropine (cholinergic- muscarinic antagonist) alone showed a significant ($p<0.05$) effect on grooming, however for rearing and locomotion the effect was insignificant compared to the vehicle respectively. While the oil alone exhibited significant ($p<0.01$- 0.001) decrease in both rearing and grooming activity in mice, except for locomotion. However, pretreatment of the animals with atropine before administration of the EOPA (150 mg/kg, i.p.) showed a significant ($p<0.01$ – 0.001) decrease in rearing and grooming, except locomotion compared to vehicle, without reversal of the inhibitory effect of the oil. These results suggest that, the inhibitory effect of the oil on rearing and grooming behaviours in mice may have been enhanced and mediated through the blockade of cholinergic transmission by the atropine (muscarinic antagonist). Therefore the oil may possibly exhibit its inhibitory effect on rearing in mice through inhibition of cholinergic transmission.

Yohimbine (α_2-adrenergic antagonist) alone showed no significant ($p>0.05$) difference on rearing, and grooming, but significantly ($p<0.001$) increased locomotion compared to the vehicle. Pretreatment with yohimbine, plus administration of the EOPA (150 mg/kg) was found to significantly ($p<0.01$ – 0.001) decreased rearing, grooming and locomotion compared to the vehicle. The antagonist was observed to enhance further the inhibitory effect of the oil on rearing, locomotion and completely abolished grooming behaviours in mice in this study. The result therefore suggests the involvement of adrenergic pathway in the mediation of the inhibitory effect of the oil through the blockade of adrenergic neurotransmission by yohimbine. Previous studies have confirmed the involvement of multiple transmitter system including acetylcholine (ACh), dopamine, serotonin (5-HT), gamma-amino butyric acid (GABA), opioid and noradrenaline in rearing behaviours in mice [22-24].

Flumazenil ($GABA_A$ antagonist) alone significantly ($p<0.05$) decreased grooming behaviour compared to vehicle, however showed no significant effect on rearing and locomotion respectively. Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system [28]. Pretreatment with flumazenil prior to administration of the oil, reversed the inhibitory effect on rearing and significantly ($p<0.001$) enhanced the effect of the oil on grooming behaviour in mice. This result is suggesting the possibility of the oil exerting its inhibitory effect on rearing through the argumentation of GABAergic neurotransmission, since flumazenil ($GABA_A$ receptor antagonist) was able to reverse the effect. However, for the grooming, the oil inhibitory effect may have been enhanced through the blockade of GABAergic transmission leading to potentiation of activity of the oil on grooming behaviour in mice. GABA and its $GABA_A$ receptors are involved in the regulation of a number of normal and pathological brain mechanisms, such as sleep, epilepsy, memory, emotions and various behaviours [27-29], including grooming.

Naloxone (opioid antagonist) also exhibited significant ($p<0.05$) decrease in grooming behaviour compared to the vehicle, however, there was no significant difference on rearing and locomotion compared to the vehicle. Pretreatment with naloxone prior to administration of EOPA, showed significant ($p<0.01$- 0.001) decrease on rearing and grooming, but not locomotion compared to the vehicle. The inhibitory effect of the oil on rearing and grooming was not reversed by naloxone. The result probably suggest that naloxone may lack significant influence on the effect of the oil, since both exert the same level of significant for both the rearing and grooming behaviours in mice.

Cyproheptadine (5-HT receptor antagonist) alone exhibited significant ($p<0.001$) decrease in grooming behaviour described as a state of low arousal in mice compared to vehicle; however the rearing and locomotion were not significantly decreased compared to the vehicle. Pretreatment with cyproheptadine prior to administration of EOPA, demonstrated a significant ($p<0.01$-0.001) decrease in rearing and grooming behaviours compared to vehicle. There was no reversal of the inhibitory effect of the oil, rather both had similar effects on rearing and grooming behaviours compared to the vehicle. The effect of the oil was also observed to have been enhanced by the blockade of the serotonergic transmission, thereby depressing grooming behaviours severely as shown with cyproheptadine plus the oil.

Furthermore, flumazenil, ($GABA_A$ antagonist) was found in this study to reverse the effect of the oil on rearing behaviour, suggesting the mediation of the inhibitory effect through GABAergic neurotransmission, corroborating its sedative activity in mice [31].

The analysis of the essential oil reveals the presence of 61 compounds out of which 51 were fully characterized and identified (Table 1) and the major ones identified include: carvacrol (8.45%), germacrene-D (8.32%), p-cimene (7.20%) and [1,1'-Bicyclopentyl]-2,2'-diol (5.97%). It is instructive to note that literature on this *P. aegyptiacus* essential oil is very scanty, However, the oil of this particular species varies considerably from the same species cultivated in Saudi Arabia in that the major compounds reported were Thymol (58.49%), γ-Terpenene (16.87%), Trans-Caryphyllene (9.11%) and α-Terpenene (7.46%) [2,35]. Comparing the chemical composition of these two oils showed variance in major compounds, hence suggesting a different chemotype with carvacrol, germacrene D, p-cymene and [1,1'-Bicyclopentyl]-2,2'-diol

6. Conclusion

This study concluded that index of acute toxicity - LD_{50} of the essential oil of *P. aegyptiacus* for both the oral and intraperitoneal routes were 2154 mg/kg (p.o) and 490 mg/kg (i.p.) respectively. The variation in the LD_{50} of the oral route compared to the intraperitoneal route may be due to hepatic first pass metabolism and other pharmacokinetic parameters. Furthermore, the results obtained in this study strongly indicated that the essential oil of *P. aegyptiacus* demonstrated significant central nervous system depressant activity, which was confirmed by the inhibition of the novelty-induced behavioural activities - rearing, grooming and locomotion in the mice. This depressant activity may have been mediated mainly through the GABAergic pathway, however, others such as cholinergic (muscarinic), adrenergic, opioidergic and serotonergic receptors or pathways may be involved to some extent. The chemical analysis of essential oil of *P. aegyptiacus* reveals the presence of about 61 compounds, out of which 51 were fully identified. The major compounds identified in the oils were carvacrol, germacrene-D, p-cimene and 1,1'-bicyclopentyl -2,2'-diol as the four prominent ones at the peak of oil profile. It cannot be predicted which of these compound (s) are responsible for the various neuropharmacological activities displayed by the oil.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declared that there is no conflict of interest

Statement of ethical approval

The animal experiments were carried out according to the protocol approved by the committee on animal use and care of the Obafemi Awolowo University, Ile-Ife, Nigeria.

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