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NEUROBEHAVIORAL ASSAY OF ETHANOL EXTRACT OF *CROTON LOBATUS* (EUPHORBIACEAE) LEAVES IN MICE

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ABSTRACT

Background: Neurobehavioural activity exhibition by an extract or compound is an indicator of the ability of its phytoconstituents to cross the blood brain barrier and modulate neurochemicals implicated in behaviours such as novelty induced behaviours, locomotion, anxiety and memory. The modulation may in part but not exclusive limited to inhibition/enhancement of enzymatic activity in the synthesis pathway of neurotransmitters or receptor blockade/mimicry activity.

Aim: To investigate the neurobehavioral properties and possible neural mechanisms of *Croton lobatus* in mice.

Methods: The neurobehavioral properties were assessed using the open field for novelty-induced rearing (NIR) and grooming (NIG), and locomotor activity in mice, while the hole board apparatus for the frequency of head dip. The Y-maze was used for short-term working memory, and the elevated plus maze (EPM) for anxiety as indexed by the open arm avoidance. Mechanistic studies were conducted with atropine, cyproheptadine, haloperidol and propranolol.

Results: The median lethal dose (LD50) of *Croton lobatus* was 1444 mg/kg, i.p. *Croton lobatus* at 5, 10, 20 and 40 mg/kg, i.p significantly dose dependently reduced novelty induced behaviors. On the Y-maze no significant change in the percentage alternation on short-term working memory was observed, while on the elevated plus maze a high index of open arm avoidance was obtained. These results confirm the central nervous system depressant properties of *Croton lobatus*, which may be mediated through α-$_2$ adrenoceptor.

Conclusion: *Croton lobatus* possess sedative property and it may be mediated through α-$_2$ adrenoceptor system.

Keywords: Neurobehavioural properties, sedation, anxiety, *Croton lobatus*.

ABBREVIATIONS: NIR: Novelty induced rearing, NIG: Novelty induced grooming, LA: locomotor activity, EPM: Elevated plus maze, HD: Head dip, EECL: ethanol extract of *Croton lobatus*, DZP: Diazepam, VEH: Vehicle, i.p: intraperitoneal, IOAA: Index of open arm avoidace

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1.0 INTRODUCTION

Croton lobatus (CL) belong to the family Euphorbiaceae and it is locally known in Nigeria as Gaásàyaá - Hausa, Òkwè –Igbo, Àjẹ́ofòlé or Eru –Yoruba. CL, a shrub, monoecious branched annual herb up to 1m tall, is widely distributed in tropical regions of the world [1]. Some countries it is distributed include Nigeria, Mexico and in China [1]. The plant is used in management of rheumatic pains, costal pains and pains from scorpion sting and bites [2-3]. Additionally, it was reported to be used in managing paralysis, eye problems, aphrodisia in males, wound healing, fever and convulsion [4-5]. However, there is dearth of information on the neurobiological properties of *croton lobatus*. Thus, the present study was designed to investigate the neurobehavioral properties of ethanol extract of *Croton lobatus* leaves.

Numerous phytochemical studies have been carried out on the *Croton* genus and revealed the presence of sesquiterpenes, diterpenes, triterpenes, steroids, flavonoids, and alkaloids [6]. Compounds isolated from the stem, bark and leaves of *Crotonlobatus*, include, steroids, diterpenes, triterpenes, polyphenols, saponims, glycosides and nitrogenous compounds [3,6]. *C. lobatus*has been found to have antiprotozoal activity [6], antibacterial [5] and antioxidant activity [3].

2.0 MATERIALS AND METHODS

2.1. Plant Materials

The leaves of *Croton lobtus* were collected in March 2019 at the Alesinoye market area, Ibadan, Oyo state, Nigeria. The taxonomical identification and authentication of the plant was done at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number 109934was deposited and compared with the reference specimen.

2.2: Preparation of Plant Material and Drugs

Air-dried leaves (50 g) were pulverized and soaked in 50% ethanol (1L) for 48 hours. The filtrate was concentrated with a rotary evaporator to give a semisolid residue and evaporated to dryness to form solid residue (6.2 g) in a desiccator. It was kept in the desiccator for further use. The dried extract was then subsequently reconstituted in distilled water at appropriate concentrations for the various experiments. All drugs and the extract were dissolved in distilled water and administered by intraperitoneal (i.p.) route.

2.3: Laboratory Animal

Laboratory animals (male Swiss mice) weighing between 18 - 25 g were obtained from the Animal House, College of Medicine, University of Ibadan, Nigeria. The animals were housed in well-ventilated transparent cages and fed with standard rodent pellet (Livestock Feed PLC, Lagos Nigeria) and water ad libitum. The United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH,

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1985) was adopted for the experimental protocol of this study.

2.4: Drugs and Chemicals

The chemicals used for this investigation are: Yohimbine (Sigma- Aldrich (St.Louis, MO, USA), Ciproheptadine, Propranolol, Haloperidol and Atropine, Naloxone (Sigma, USA).

2.5: Experimental Procedures

2.5.1: Acute toxicity test

The method described by Lorke (1983) was used to determine the LD_{50}, which is the index of acute toxicity. Male Swiss Albino mice (20 - 25 g) were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals. Ethanol extract of *Croton lobatus* was administered intraperitoneally (i.p.) (10, 100 and 100 mg/kg), a dose for each group. The treated animals were monitored for 24 hours mortality and general behaviour. From the results of the above step, four different doses of (500, 1000, 2000 and 3000 mg/kg) were chosen and administered intraperitoneally respectively to four groups of one mouse per group. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

2.5. Neurobehavioral Assays

2.5.1. Novelty-induced rearing (NIR) and grooming (NIG) in mice

The behavioural profiles of EECL on novelty-induced exploratory behavioural phenotypes (rearing and grooming). Rearing was taken as the number of times the mouse was standing on its hind limbs or with its forelimbs against the wall of the observation cage or in the free air while rooming was taken as the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions [8-9]

2.5.2. Exploratory behaviour on hole-board apparatus

The effect of EECL on the number of head-dips; a measure of CNS excitability or sedation was evaluated using the hole-board in non-aversive conditions as previously described [10]. The evaluation was based on the frequency of head-dips, though this can be a means of escape in aversive conditions [14]. The number of times that each animal dipped its head into the holes was counted for the period of 5 min [10].

2.5.3. Locomotor activity in the open field

Effect of EECL on locomotor behaviour was assessed using the open field method as described previously [11]. This assessment was based on the number of lines the animal crossed in 5 min. Ethanol (70%) was used to clean the activity cage at the end of each session to prevent odour bias[11].

2.5.4. The Y maze test

The behavioural effect of EECL on spatial working memory was assessed using the Y-maze as described previously [17]. The evaluation was based on correct percentage alternation.An alternation is defined as an entry into all three arms on consecutive devices. Percentage alternation was calculated as the ratio of actual alternations to possible alternations (defined as the total number of arm entries minus two) multiplied by 100. Ethanol (70%) was used to clean the Y-maze at interval [17].

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2.5.5. Elevated plus Maze Test

The neurobehavioral effect of EECL on possible anxiolytic effect was evaluated using EPM as described by Lister [13]. The evaluation based on the time spent particularly on the open arm (i.e open arm entry) during this test period. Arm entry is described as entry with both fore- and hind-limbs into one arm in this experiment. Ethanol (70%) was used to clean the maze at the end of each session to prevent odour bias. The Index open arms avoidance [IOAA] was determined i.e. IOAA= 100 - (% time spent in open arms + % entries into open arms)/2.

2.5.6. Mechanism of Action

In another set of experiment conducted, mice were pre-treated (intraperitoneally) 15 minutes prior with neurotransmitter blockers to evaluate the possible mode of actions of the extracts on novelty-induced behaviours in mice. The following transmitter receptor blockers were used: atropine (muscarinic blocker, 0.5 mg/kg), naloxone (opioid receptor blocker, 0.25 mg/kg), propanolol (non-selective ß-adrenoceptor blocker, 0.2 mg/kg), haloperidol (D_2 receptor blocker, 0.2 mg/kg), ciproheptadine (Serotonine antagonist, 0.5 mg/kg) and yohimbine ($ά\text{-}_2$ adrenergic blocker, 1 mg/kg) [14].

2.5.7. Statistical analysis

All data were presented as Mean ± SEM. The results were analyzed by One way analysis of variance (ANOVA) and post hoc tests (Student's-Newman-Keuls) were carried out to determine the source of significant main effect using GraphPad InStat® Biostatistics software. The level of significance for all tests was set at $P < 0.05$.

3.0 RESULTS

Toxicity test

The median lethal dose (LD_{50}) of *Croton lobatus* crude extract in mice was found to be 1444 mg/kg i.p. body weight. This determination was done in a 48 hours continuous observation.

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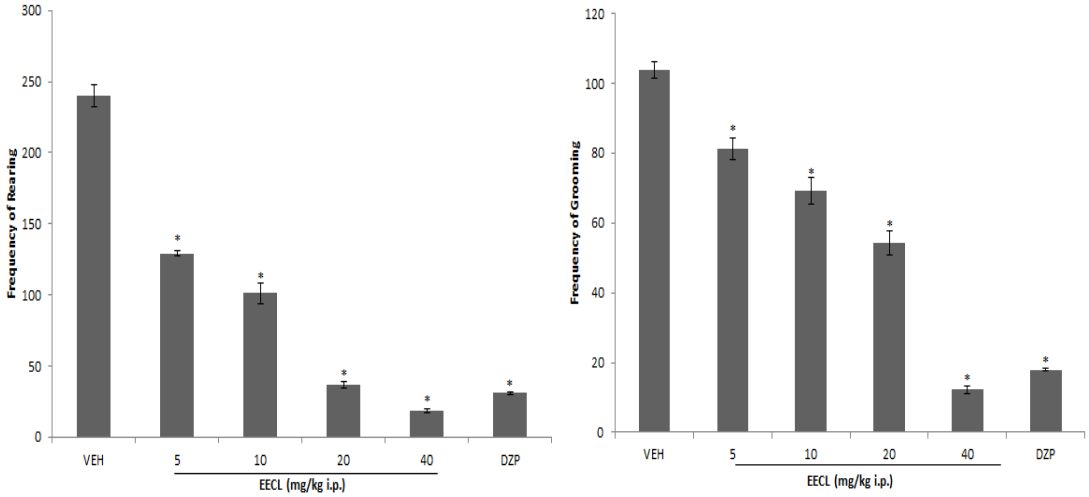

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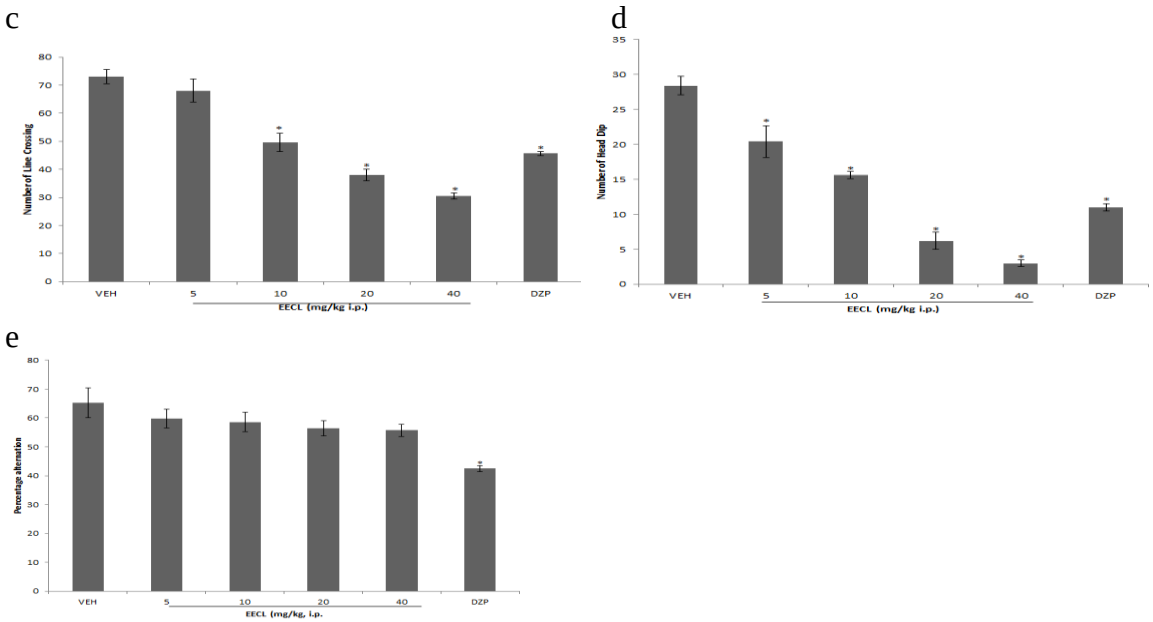

Figure 1: (a). Effect of EECL on novelty-induced rearing (NIR) [F = (5, 24) = 345.2, (P < 0.0001)], (b) novelty-induced grooming (NIG) [F = (5, 24) = 213.9, (P < 0.0001)], (c) locomotor activity in open field [F (5, 24) = 41.80 (P < 0.0001)], (d) frequency of Head dips in hole-board [F(5, 24) = 56.87 (P < 0.0001)], (e) learning and memory in the Y-maze , [F (5, 24) = 6.89 P < 0.0001] in mice. The results are presented as Mean ± SEM (n= 5). Data was analysed statistically analysis using One way ANOVA. * Indicates significant difference from the control P < 0.05 (Student-Newman-Keuls test). VEH: Vehicle; EECL: Ethanol Extract of *Croton lobatus;* DZP: Diazepam (2 mg/kg, i.p).

The effect of EECL on arm entry (open and close arm), percentage duration of arm entry and index of open arm avoidance; a measure of anxiolysis was evaluated using the aforementioned parametersin the elevated plus maze [11]. The evaluation based on the time spent particularly on the open arm (i.e open arm entry) during this test period. Arm entry is described as entry with both fore- and hind-limbs into one arm in this experiment. All doses of showed no statistical significance in percentage open arm duration and a high index of open arm avoidance. In contrast, diazepam, the standard, showed a statistical significance in open arm entry time and a reduced index of open arm avoidance as indicated in Table 1.

Table 1: Effect ethanol extract of Croton labatus on mice in the Elevated-Plus Maze (EPM)

GROUP	Dose (mg/kg)	Open Arm Entry	Closed Arm Entry	% Open Arm Entry	% Open Arm Duration	Index of Open arm Avoidance
VEH	10ml/kg	0.80 ± 0.37	8.40 ± 0.25	9.10 ± 1.11	3.20 ± 0.45	93.61 ± 2.11

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EECL	5	0.40 ± 0.25	5.00 ± 0.32*	7.69 ± 1.21	1.10 ± 0.11	95.80 ± 1.44
	10	0.20 ± 0.20	5.40 ± 0.40*	3.60 ± 0.77	0.80 ± 0.09	97.80 ± 1.26
	20	± 0.00	3.60 ± 0.51*	0.0 ± 0.00	0.0 ± 0.00	100.00 ± 1.11
	40	± 0.00	2.60 ± 0.25*	± 0.00	± 0.00	100.00 ± 1.34
DZP	1	7.80 ±1.39*	1.80 ± 0.37*	20.00 ± 2.22*	80.40 ± 4.57*	18.23 ± 2.59*

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is no significant [F (5, 24) = 62.08, P > 0.05] difference between the control and treatment groups. Although significance is observed in closed arm entry compared to the control. The standard drug also revealed significance in all paradigms compared with the control group.
* Indicates significant difference from the control P < 0.05 (Student-Newman-Keuls test).
VEH: Vehicle; EECL: Ethanol Extract of *Croton lobalus;* DZP: Diazepam (1 mg/kg, i.p.).

The effect of antagonists of various systems such as cholinergic (atropin), serotonergic (cyproheptadine), dopaminergic (haloperidol), opioid (naloxone), beta-adrenergic (propranolol) and alpha-adrenergic (yohimbine) systems on novelty induced grooming, rearing, head dip and locomotor activity in drug naïve experimental animals (mice). Statistically significant reduction in rearing, grooming, head dip and locomotor activity were observed for all the antagonists employed except for atropine that showed no significance hole-board apparatus comparable with the vehicle treated groupas indicated in Table 2.

Table 2. Effect of Atropine, Cyproheptadine, Haloperidol, Naloxone, Propranolol and Yohimbine on NIR, NIG, HD and LA in mice

Group	Dose (mg/kg)	Rearing	Grooming	Head Dip	Locomotor Activity
VEH	10ml/kg	240.2 ± 7.83	103.80±2.46	28.40 ± 1.33	73.00 ± 2.55
Atropine	0.5	128.80 ± 8.42*	40.60 ± 2.56*	32.20 ± 2.33	58.60 ± 2.42*
Cypreheptadine	0.5	103.0 ± 3.97*	17.20 ± 1.36*	12.60 ± 1.36*	46.0 ± 2.61*
Haloperidol	0.2	107.06 ± 11.64*	46.00 ± 0.70*	13.00 ± 0.71*	20.00 ± 1.23*
Naloxone	0.25	81.12 ± 2.12*	29.50 ± 2.33*	15.42 ± 2.51*	48.44 ± 5.33*
Propranolol	0.2	96.00 ± 4.03*	42.60 ± 5.41*	19.40 ± 1.50*	34.20 ± 2.27*
Yohimbine	1	127.20± 14.39*	38.20 ± 7.94*	11.20 ± 0.86*	40.20 ± 3.88*

Results are expressed as mean ± S.E.M, (n=5). Statistical analysis on One way analysis of variance (ANOVA) showed a significant difference [F (5, 24) =17.74, $p < 0.0001$ (NIR); F (5, 24) = 21.44, $p < 0.0001$ (NIG); F (4, 24) = 35.13, $p < 0.0001$ (HD)] and LA [F (4, 24) = 20.68, $p < 0.0001$] in between control and treatment groups. * Indicates significance between control and treatment groups, $p < 0.05$ (Student-Newman-Keuls test).NIR: Novelty-Induced Rearing; NIG: Novelty-Induced Grooming; HD: Head Dips; LA: Locomotor Activity.

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The effect of pretreatment of antagonists of various systems such as cholinergic (atropine), serotonergic (cyproheptadine), dopaminergic (haloperidol), opioid (naloxone), beta-adrenergic (propranolol) and alpha-adrenergic (yohimbine) systems on novelty induced grooming, rearing, head dip and locomotor activity in drug naïve experimental animals (mice) and its mechanistic inhibitory effect ethanol extract of *Croton lobatus*. A statistically significant reversal in rearing, grooming, head dip and locomotor activity were observed due to blockade of adrenergic (α and β adrenoceptors; haloperidol, Yohimbine and propranolol). Similar significant reversals were observed for cholinergic (head dip), serotonergic and opioid antagonists pretreated groups. as indicated in Table 3.

Table 3. Effect of pre-treatment with Atropine, Cyproheptadine, Haloperidol, Naloxone, Propranolol and Yohimbine on the inhibitory effect of the ethanol extract of Croton lobatus on NIR, NIG, HD and LA in mice

GROUP	Dose (mg/kg)	Rearing	Grooming	Head Dip	Locomotor Activity
VEH	10ml/kg	240.2 ± 7.83	103.80±2.46	28.40 ± 1.33	73.00 ± 2.55
EECL	40	36.60 ± 2.40*	54.20 ± 3.46*	6.20 ± 1.24*	38.00 ± 2.07*
Atropine + EECL	0.5 mg/kg	24.00 ± 1.90	43.00 ± 1.67	21.20 ± 0.80**	35.80 ± 1.24
Cyproheptadine + EECL	0.5 mg/kg	± 0.00*	± 0.00*	± 0.00*	31.22 ± 2.22
Haloperidol + EECL	0.2 mg/kg	24.20 ± 1.43	29.80 ± 1.63*	6.40 ± 0.93	44.40 ± 1.92
Naloxone + EECL	0.25 mg/kg	166.32 ± 4.72**	69.75 ± 3.30	24.33 ± 1.22**	64.52 ± 3.62**
Propranolol + EECL	0.2 mg/kg	29.80 ± 1.02	34.00 ± 1.70*	11.00 ± 1.05	40.40 ± 3.14
Yohimbine + EECL	1 mg/kg	63.40 ± 4.23**	61.60 ± 4.92	38.80 ± 1.32**	51.20 ± 2.60**

Results are presented as mean ± S.E.M, (n = 5). Pre-treatment with Naloxone and Yohimbine reversed the inhibitory effect of extract of *C. lobatus* on novelty-induced rearing (NIG) and grooming (NIG), HD and LA. One way analysis of variance (ANAVO) showed a significant difference [$F(6, 28) = 184.30$, $p < 0.0001$ (NIR); $F(6, 28) = 76.10$, $p < 0.0001$ (NIG); $F(6, 28) = 186.1$, $p < 0.0001$ (HD)] and LA [$F(6, 28) = 32.29$, $p < 0.0001$] in between EECL + Antagonists and EECL alone.

* Indicate significant difference (further depression) from the EECL, $p < 0.05$ (Student-Newman-Keuls test).

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** indicates significant difference (reversal) from the EECL. $p < 0.05$ (Student-Newman-Keuls test). Novelty-induced rearing (NIG) and grooming (NIG), HD: Head dips, LA: Locomotor activity.

DISCUSSION

The growing interest in alternative medicine (medicinal plants) have instigated a compelling need for toxicity studies in medicinal plants and or natural products. It's even of greater concern particularly in African countries such as Nigerian where the health care system is still evolving and availability of essential western medicine in the rural communities does not equilibrate demand [17]. Hence, the dependence on medicinal plants and other natural products in the management of common ailments.

Animal models have been used over time to extrapolate toxic effect of toxins, chemicals and medicinal products to humans. The acute lethal dose of the EECL was calculated to be 1444 mg/kg. This suggests that the higher the Lethal Dose value for a substance, the relatively safe the substance is presumed to be [14]. The LD_{50} value of *Croton lobatus* in this study is suggestive of its safety, more so, the different doses selected for this study was informed on the lethal dose value.

Neurobehavioral profile of an extract or a compound may in part but not exclusively influenced by central nervous system excitability or sedation [14]. This may be achieved through interference on the enzyme system involved in the synthesis of a neurotransmitter (dopamine or GABA) [17], the process of release of the transmitter into the synaptic cleft, enzymatic system responsible for the breakdown of the transmitter and on the receptor system that the transmitter binds to elicit its physiologic effect [17-18]. Rearing a vertical locomotive activity involves an animal standing on its hind limbs while raising up with its forearms in the air or placed on the wall of the cage [9]. This is an explorative behaviour, which evaluates central nervous system excitation [15]. Evaluations from this behavioural phenotype has been employed to categorize test drugs/substances as stimulants or sedatives [16]. Lines of evidence [17] have shown that CNS stimulants enhance rearing while CNS depressants have been linked to inhibition of this behaviour [18]. The results obtained shows that EECL significantly reduced rearing behaviour dose dependently, indicative of inhibitory effect on novelty induced rearing(NIR) behaviour and suggestive of sedative activity.

Grooming is described as face or head cleaning, maintenance and hygiene behaviour in animals' imperative in restoring homeostasis under frustrated and stressful condition [12, 19]. Inhibition of this behaviour is suggestive of the ability of an extract or substance to reduce stress in experimental animals. Results obtained from this study shows a significant reduction in grooming dose dependently on intraperitoneal administration of administration of ethanol extract of *Croton lobatus*. The inhibitory effect observed at 40 mg/kg was comparable to the standard, diazepam.

This inhibitory effect observed in both novelty-induced rearing (NIR) and grooming (NIG) behaviours suggest the

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ethanol extract of *Croton lobatus* possess a sedative or central nervous system depression activity. This study agrees with some plants that have been shown to possess strong sedative effects such as *Cryptolepsis sanguinolenta, Cissus quadrangulensis, Spondi amombin, Ficus thoningii, Nigella sativa* and *Stachys lavandu folia* [20-25].

Central nervous system excitation or exhibition by an extract or compound is an indicator of the ability of phytoconstituents in the extract or compound to cross the blood brain barrier and modulate neurochemicals implicated in behaviours such as exploratory behaviour in experimental animals [17-18]. In the hole-board, exploratory activity is depicted by increase in the number of head-dips into the holes in non-aversive condition while a reduction in the number of head-dips indicates dominance of inhibitory activity, however, in aversive condition, poking of the head of animal into the holes is to aid escape [17]. Hence, this behavioural test is employed to screen sedative extracts or compounds in experimental animals [26-27]. In this study EECL significantly in a dose dependent fashion reduced the number of head-dips in the hole-board apparatus, suggestive of potentiation of the inhibitory system [17]. This study is in agreement with File and Wardill [26] report, that agents whose effect cause a reduction in the frequency of head dips depicts CNS depression. Moreover, the hole-board apparatus can also be employed to evaluate anxiety and assess anxiolytic compounds. In the use hole-board to evaluate anxiolysis, it is presumed that head-dipping of animals is inversely proportional to their anxiety state in moderately aversive environment [14]. Consequently, an increased in the number of head dips on the board portrays reduced anxiety state [28]. In aversive conditions, the holes may present possible routes of escape instead of exploration and the relationship between anxiety state and head-dipping activity has been reported not to be inverse but directly proportional [28]. Literature search revealed that anxiolytics augment the frequency of head poking, whereas sedative agents reduce the frequency of head poking [17,18 and 28]. The observation that EECL significantly reduced the frequency of head-dips dose dependently further suggest that *Croton lobatus* possess sedative activity [17].

Spontaneous motor activity is a parameter used to measure the central nervous system excitability or inhibition in animals [17]. Locomotor activity either enhancement or inhibition is a strong predictor of central nervous system activity [28]. The predominance of excitatory pathway is predicted by an increase in number of lines crossed in the open field, while a reduction in the number of lines crossed indicates a takeover of the inhibitory pathway [17-18]. Motor activity has been shown to be primarily governed by motor area of brain stem, corpus striatum and frontal cortex [29]. Morphological changes or change in neurochemical levels (amines) in these brain regions is likely to cause neurotoxic effect, which may present as motor deficit[29]. Interestingly, dopamine has been reported as the predominant aminergic neurotransmitter identified in these brain regions and has been implicated in exploratory and locomotor activity [17-19]. Studies have shown that hypodopaminergic activity in adult animals produces decreased

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spontaneous activity [30] whereas, dopamine receptor antagonists (haloperidol) have been shown to impair locomotor activity [31]. Consequently, dopaminergic transmission enhancing agents have been observed to argument locomotor activity [12,17] and are termed central nervous system stimulants. The agents suppress dopaminergic transmission attenuates locomotor activity, hence are termed central nervous system depressants [14,17]. The present study shows that EECL produced a significant reduction in locomotor activity at the higher doses in a dose dependent manner nevertheless at the lowest dose the reduction was not significant. Therefore, the decrease in locomotor activity with the three higher doses of the ethanol EECL may be an index of the central nervous system depressant effect of the extract.

The Y-maze is a behavioural apparatus used to evaluate spatial working memory of experimental animals extrapolative of cognitive function in humans [17]. Spontaneous alternation assesses the inclination of an experimental animal to explore new environments as laboratory animals characteristically prefer to explore a new arm of Y-maze rather than returning to one that was previously visited [17-18]. Memory is a highly complex process that involves several neurotransmitters and neuropeptides [12,32]. This test is used to quantify cognitive deficits in transgenic strains of mice and evaluate novel chemical entities for their effects on cognition. *Croton lobatus* at the doses used for experiment has no effect on working memory as the percentage alternation produced is not significant from the control.

The responses observed upon evaluation of novelty-induced behaviours such as rearing and grooming have been demonstrated to involve multiple neurotransmitter system; dopamine, opioid and GABA receptors [4,32]. Inhibition or attenuation of novelty induced rearing behaviours have been linked to potentiation of the inhibitory pathway (GABA) and inhibitory activity on the excitatory pathway (glutamate, dopamine, systems) [11,14]. Studies have demonstrated that grooming behaviour in experimental animals is connected with dopamine receptor, particularly in the mesolimbic region of the brain [34]. Lines of evidence have shown that D_1-like (D_1 and D_5) receptor antagonists attenuates grooming behaviour; suggesting the involvement of D_1-like receptors in grooming activity [11,14,34].

Furthermore, the study investigated the neuronal pathway EECL exerts its behavioural effect by interaction with antagonists of pathways that influence behaviours in experimental animals. Administration of atropine, cyproheptadine, haloperidol and propranolol showed no significant reversal of EECL induced inhibition of grooming and rearing. Contrastingly, cyproheptadine potentiated the inhibitory effect of EECL on grooming and rearing. A separate experiment with yohimbine demonstrated a significant reversal of the behavioural effect of EECL on grooming and rearing. The trajectory of the above experiments suggests the exclusion of cholinergic, serotonergic, dopaminergic, ß-adrenergic and histaminergic pathways. Accordingly, the experiment with yohimbine suggest that EECL may contain some phytoconstituents

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with affinity for α_2-adrenoceptor, indicative of the involvement of α-noradrenoceptor pathways in EECL behavioural activity.Correspondingly, pre-treatment with Naloxone significantly reversed the effect of EECL in novelty-induced grooming and rearing, and locomotor activity. This suggests the implication of the opiate receptors in the inhibitory effect elicited by EECL.

5. CONCLUSION

The inhibitory activity of EECL demonstrated on novelty-induced neurobehavioral phenotypes suggest that EECL possess phytoconstituents with sedative or central nervous system depressive activity; indicative of potentiation of inhibitory pathways or inhibitory effect on the excitatory pathway. However, the mechanistic study suggests the involvement of α-$_2$ adrenoceptors and opioid receptors.

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Ethical Considerations

Experiments were approved and carried out according to the guidelines of the University of

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Ibadan's Animals Ethic Committee (Ethical number: UI-ACUREC/17/0111) in stern compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication number: 85-23, revised 1985).

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Author Contributions: Aya-Ebi O.E and Ben-Azu B. conceived the concept of the research. Felicia O.O was involved in the analysis of collected data while Aderibigbe O.E supervised the work.All authors are accountable for the entire content of this manuscript and approved the submission.

Competing interests:
All authors read through this manuscript, effected corrections submitted and approved the publication of this manuscript