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EFFECTS OF ETHANOLIC EXTRACT OF *DIOSCOREA BULBIFERA* BULB ON NEUROBEHAVIOURAL PARAMETERS (LEARNING AND MEMORY) IN THE HIPPOCAMPUS OF THE BRAIN OF MALE WISTAR RATS

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ABSTRACT

The aim of the study was to determine the effects of ethanolic extract of *Dioscorea bulbifera* bulb on neurobehavioural parameters (learning and memory) in the hippocampus of the brain of male Wistar rats. The rats were divided into four (4) groups, designated A, B, C and D. They contained 8 rats each. GROUP A: (Control): - The control rats had access to food and water only and was given no extract. GROUP B: (Low dose): - Male Wister rats were given a low dosage of 150mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day. GROUP C: (Medium dose): - The male Wister rats were given a medium dosage of 300mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day. GROUP D: (High dose): - Male Wister rats were given a high dosage of 600mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day. Data was analysed using SPSS version 22. ANOVA of variance test was conducted followed by post Hoc LSD comparison and values were considered significant at $p \leq 0.05$. The result showed a significant increase in the number of entries in open arm in group D ($p=0.019$) compared to A. The time spent in open arms showed a statistically significant increase in group D ($p=0.026$) compared to A. The time spent in closed arm showed a statistically significant decrease in groups C, and D ($p=0.001$, $p=0.001$) compared to group A, but group B showed no significance ($p=0.341$). The result showed a non-significant decrease in the no. of trials in groups B, C, and D ($p=0.113$, $p=1.000$, $p=0.259$) compared to group A. The latency period revealed a significant decrease in groups B, C, and D ($p=0.039$, $p=0.033$, $p=0.020$) compared to group A. In conclusion, the present study demonstrates that ethanolic extract of *Dioscorea bulbifera* possesses anxiolytic-like activity in Wistar rats, with the most pronounced effect observed at the high dose.

KEYWORDS: Ethanolic extract, *Dioscorea bulbifera*, hippocampus, neurobehavioural parameters, learning and memory, Wistar rats.

1. INTRODUCTION

The Dioscorea species, a climbing herb with rhizome-producing roots, is a member of the Dioscoreaceae family and is widely cultivated across tropical and subtropical regions worldwide. While Africa accounts for a staggering 96% of global production, other areas like South America, the Caribbean, and Southeast Asia also cultivate Dioscorea, making them significant secondary hubs for its production. (Anand *et al.*, 2011).

The Dioscorea tubers are present at all seasons of the year and have a 3m (10ft) height. The stems (rhizomes) are oblique and arranged at irregular intervals. The leaves are estimated to have a length of 5-11.5cm, width of 4-10.5cm, triangular oval-shaped, often cordate-shaped, spiked, both sides smooth and globous, petiole slender, 5-10cm long. On the aspect of the leaf axils, male flowers are simple or sometimes branched, slim, flexible, 7.5-25cm long. Flowers tiny, secluded in clusters; stamens 6, anthers inferior. Female flowers stalk solitary, slender up to 15cm long. Underground rhizomes of Dioscorea are commonly known as wild yam (Anand *et al.*, 2011).

As observed, Dioscorea is of very high importance in private companies, for instance, in the pharmaceutical industries, and the plant is mainly collected in India along with Himalaya (Mudasir, 2012, Rawat, *et al.*, 2011). Dioscorea is endangered in natural habitats due to illicit use, spread of cities, and over-use by local peoples for trading and domestic purposes, (Gopichand *et al.*, 2013). In Indian Himalayan regions, it is found in Arunachal Pradesh, Sikkim, Assam, Meghalaya, Jammu and Kashmir, Himachal Pradesh, and Uttarakhand (Anand *et al.*, 2011). The plant's rhizomes have the presence of Diosgenin, which makes it special; the diosgenin is a phytoestrogen which converts into the hormone progesterone. Diosgenin can serve as the basis for anti-fertility drugs such as contraceptive pills, and sex hormones, such as testosterone, builders use this as a supplement to build muscle strength as well as boost their testosterone levels.

Dioscorea tubers are straight and zinger-like shaped. *Dioscorea* is used in the management of several diseases such as gastrointestinal disorders, sore throat from struma, diarrhoea, irritability, abdominal pain, wounds, burns and anaemia. The tubers are likely to have some activities like antimicrobial, antioxidant, stomachic and hypoglycaemic activities (Subhash *et al.*, 2012), adding to it, dysentery, piles and chronic liver pain diseases have been extremely being treated with Dioscorea (Dangwall *et al.*, 2013).

Neurobehavioral evaluations are very important to ascertain any neurotoxicity of neurotoxins. Such evaluations are related to the examination or analysis of the central nervous system, i.e., the study of the mind because the mind is the living state of the brain (Kumar, 2023).

2. MATERIALS AND METHODS

2.1 Study Location

This study was conducted at the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

2.2: Procurement and Identification of Plant

Dioscorea bulbifera was gotten from Eke Amobi Central market, Nnewi, in Anambra State, Nigeria. It was identified and authenticated by the Taxonomist in the Department of Biological Sciences, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Anambra.

2.3: Method of Extraction

Fresh bulbs of *Dioscorea bulbifera* was bought from Eke Amobi Local market in Nnewi, Nnewi North Local Government Area of Anambra state. The seeds were washed, sliced and dried. The dried seed was grounded into a coarse form with a local grinder. 250mls of the grounded *Dioscorea bulbifera* bulb was macerated in 1000mls of 98% Absolute Ethanol (BDH England) for 48hours. After 48hours, the mixture was sieved using a porcelain cloth and was further filtered using Whatman no 1 filter paper into a clean glass beaker, and the filtrate was concentrated using Digital Rotary Evaporator (TT-55 Techmel $ Techmel USA) and was further dried using a Thermostat Oven (DHG 9023A PEC Medicals USA) into a paste like form and stored in Refrigerator (Nexus).

2.4: Phytochemical Analysis using the HPLC procedure

The ethanolic extract of *Dioscorea bulbifera* contains Dodecane, Hexadecane, 5-Tetradecene, (E)-Cetene, 4-Tetradecene, (Z)-Cetene, Tetradecane, Cyclododecane, 2,4-Di-tert-butylphenol, Undecanoic acid, 10-methyl-, methyl ester, Dodecanoic acid, and 9-Octadecene, (E)-Cetene. Also indicated in the extract were Cyclohexane, 1,1'-(1,4-butanediyl) bis-Cyclohexane, butyl-Cyclohexane, Cyclotetradecane 2-Tetradecene, 1-Dodecene, Heptadecane, Tridecanoic acid, 12-methyl-, methyl ester, 1,2,4-Butanetriol, Tetradecanoic acid, n-Hexadecanoic acid, Palmitoleic acid, Oleic Acid, amongst others.

2.5 Lethal Dose (LD-50)

The median Lethal Dose (LD50) of *Dioscorea bulbifera* ethanoic extract, was carried out in the Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus. This was this was determined using the method of Lorke, (1983) in this study. 12 Rats were used. They received the extract via oral route, and it was carried out into two phases.

A total of nine (9) rats were employed for the study and three (3) were allocated for each group.

PHASE I
Group 1 received 10mg/kg per rats.
Group 2 received 100mg/kg per rats
Group 3 received 1000mg/kg per rats

The animals were monitored for 24hrs for morbidity and mortality. The rats remained normal after 24hrs of observation in phase 1. Then the study proceeded to the second phase, where three (3) rats were employed for the study comprising one (1) rat per group.

PHASE II
Group 1 received 1200mg/kg per rat
Group 2 received 1600mg/kg per rat
Group 3 received 2900mg/kg per rat
Group 4 received 5000mg/kg per rat
A
The animals were monitored for another 24hrs for mortality and morbidity.
LD-50= a*b
A= Maximum dose with 0% mortality
B= Minimum dose with 100% mortality
LD50= a*b
LD50 if ethanolic extract of *Dioscorea bulbifera* is above 5000mg/kg

2.6: Animal Source
Forty (40) male wister rats weighing 70-90kg, were purchased from the Animal house of the Department of Physiology, Nnamdi Azikiwe University, Okofia Campus Nnewi.

2.7 Procedures for Acclimatization, Feeding and Administration of Extract

- **Acclimatization**

The animals were bred at the Animal House of the Department of Physiology, Chukwuemeka Odumegwu Ojukwu University, Uli campus, under standard condition. Twelve (12) hours daylight and Twelve (12) hours darkness, good humidity and ventilation in a wooden cage containing sawdust bedding and were allowed to acclimatize for two (2) weeks.

- **Feeding**

The forty (40) rats were fed with starter feed firstly to help their growth and continued with grower feed mesh; they were also provided with tap water.

- **Administrations of Extract**

Oral administration was performed using cannula determine their dosage.
Group A Control: This group was given no extract they had free access to food and water.
Group (B, C and D): - where feed Extract for Twenty-eight (28) days.
During the weeks of extracts administration, we noticed that the extracts make them weak and hungry and less thirsty. No loss was recorded, and they lost weight after the two weeks of administrations especially the High dose group (Group D).

$$\frac{\text{Average weight of animal (kg) x Dose (mg/kg)}}{\text{Stock (mg/ml)}}$$

10g of gel-like extract of *Dioscorea bulbifera* was dissolved in 100ml Group B male [Low dose 150mg/kg]

$$=. \frac{0.102 \times 150\text{mg/kg}}{100\text{(mg/ml)}} = 0.2\text{ml}$$

Group C [Medium dose 300mg/kg]

$$=. \frac{0.12\text{kg} \times 300\text{mg/kg}}{100\text{(mg/ml)}} = 0.4\text{ml}$$

Group D [High dose 600mg/kg]

$$=. \frac{0.14\text{kg} \times 600\text{mg/kg}}{100\text{(mg/ml)}} = 0.84\text{ml}$$

After two weeks of the administration, we observed that the *Dioscorea bulbifera* bulbil extract given to the animals in the three test groups:

- There was increase in taste.
- Immediately after administration of extract, they were calm.

After the extract has digested, they become normal.

2.8. Experimental Design
The rats were divided into four (4) groups, designated A, B, C and D. They contained 8 rats each.
GROUP A: (Control): - The control rats had access to food and water only and was given no extract.
GROUP B: (Low dose): - Male Wister rats were given a low dosage of 150mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day.
GROUP C: (Medium dose): - The male Wister rats were given a medium dosage of 300mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day.
GROUP D: (High dose): - Male Wister rats were given a high dosage of 600mg/kg body weight of ethanolic extract of *Dioscorea bulbifera* twice a day.

2.9 Y-Maze Test
Y-Maze test is based on the natural drive of rodents to explore novel environments and test the short-term spatial recognition memory. This test requires neither food deprivation (unlike the radial maze) nor electrical foot-shock (unlike the light/dark box), which could modify the motivational and emotional status of the animal. The Y-maze test was successfully applied to study the effect of age, administration of various drugs and food supplements, as well as various stressors, on spatial memory performances.

The Y-maze consists of three identical arms mounted in the shape of a "Y." Each arm is usually 15 inches long and 3.5 inches wide with 3-inch-high walls made of opaque material. The process consists of two different sessions, each about 30 minutes interval. During the first session, which is 15 minutes interval, one arm (the novel arm) is blocked but the mouse can examine the other two

open arms. While in the second session, another 15 minutes all arms are open. The position of the animal in the maze is usually documented by a tracking system. The parameters to be visualized are: the first arm entered, number of entries into different arms, and time spent in each arm. The dependent variable is time spent in the novel arm of the Y-maze. During the second session, mice with steady spatial memory will usually make the first turn into the novel (i.e., previously blocked arm) and spend approximately 60%–70% of the time in that arm.

2.10 Elevated Plus Maze/T-maze

The EPM is a rodent model of anxiety that has been used broadly in the characterization of both established drugs and new chemical objects for anxiolytic activity. It is an etiologically based test that uses non painful, non-aversive stimuli to induce fear and anxiety thus reducing the possible confounds of motivational and perceptual states. The test is an adjustment of the Y-maze test that depend on the tendency of a rodent to spend less time in the open areas of the maze than the closed areas. A typical apparatus is the shape of a 'plus' sign and has two elevated arms, one open and one closed. The center of the maze is an open area, and the mice is placed on this center area at the beginning of the test. Over the course of a 5 min duration, the period the animal spends in either the open or closed areas is documented. Since rodents have an inborn fear of height and openness untreated/naive animals spend less time on the open versus closed arms, and anxiolytics like diazepam will increase the amount of time spent in the open arm showing a decrease in the anxiety of the animal.

2.11: Ethical Clearance

Ethical approval was given by the Research Ethics Committee, Faculty of Basic Medical Science, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. The National Institutes of Health's recommendations for the management and care of laboratory animals were followed when handling and treating rats.

2.12: Data Analysis

Data was analysed using SPSS version 22. ANOVA of variance test was conducted followed by post Hoc LSD comparison and values were considered significant at $p \leq 0.05$.

3.0 RESULTS

Table 3.1: Effect of ethanolic extract of *Dioscorea bulbifera* on anxiety level using elevated maze plus.

	No. of entries in open arm	No. of entries in closed arm	Time spent in open arm (seconds)	Time spent in closed arm (seconds)
	MEAN±STD	MEAN±STD	MEAN±STD	MEAN±STD
Group A (control)	0.80±0.44	1.60±0.89	7.40±3.02	114.80±4.36
Group B (150 mg/kg of EDB)	1.00±0.81[a]	2.00±1.15[a]	11.50±3.83[a]	100.75±10.04[a]
Group C (300 mg/kg of EDB)	1.20±1.09[a]	1.61±0.79[a]	15.40±8.26[a]	57.60±13.51[**]
Group D (600 mg/kg of EDB)	2.33±1.21[**]	2.83±0.16[a]	29.00±7.38[**]	63.33±8.28[**]
p-value	**0.05**	**0.14**	**0.06**	**0.02**
F-ratio	**2.81**	**1.76**	**2.31**	**8.65**

Data was analysed using ANOVA followed by post Hoc LSD comparison and values were considered significant at $p \leq 0.05$. EDB: ethanolic extract of *Dioscorea bulbifera*, **= significant, a= not significant.

The result showed an increase in the number of entries in open arm in groups B, C, and D (*p=0.762, p=0.522, p=0.019*) compared to A, but groups B and C had no significance and group D had significance. The number of entries in close arm revealed a a non-significant increase in the number of entries in groups B, C, and D (*p=0.574, p=1.000, p=0.067*) compared to A. The time spent in open arms showed an increase in groups B, C, and D (*p=0.679, p=0.396, p=0.026*) compared to A, but groups B and C had no significance and group D had significance. The time spent in closed arm showed a decrease in groups B, C, and D (*p=0.341, p=0.001, p=0.001*) compared to group A, but group B showed no significance and groups C and D had significance.

Table 3.2: Effect of ethanolic extract of *Dioscorea bulbifera* on spatial memory using Y-maze.

	No. of Trials	Latency (%)	No. of errors
	MEAN±STD	MEAN±STD	MEAN±STD
Group A (control)	2.40±0.89	12.24±1.75	0.40±0.24
Group B (150 mg/kg of EDB)	1.50±0.57[a]	9.57±1.72[**]	0.25±0.25[a]
Group C (300 mg/kg of	2.31±0.89[a]	9.62±1.65[**]	0.60±0.24[a]

EDB)			
Group D (600 mg/kg of EDB)	1.83±0.75[a]	9.46±1.89[**]	0.50±0.22[a]
p-value	**0.41**	**0.00**	**0.32**
F-ratio	**1.42**	**2.89**	**0.34**

Data was analysed using ANOVA followed by post Hoc LSD comparison and values were considered significant at $p \leq 0.05$. EDB: ethanolic extract of *Dioscorea bulbifera*, **= significant, a= not significant.

The result showed a non-significant decrease in the no. of trials in groups B, C, and D (*p=0.113, p=1.000, p=0.259*) compared to group A. The latency period revealed a significant decrease in groups B, C, and D (*p=0.039, p=0.033, p=0.020*) compared to group A. The no of errors showed a decrease in group B (*p=0.684*), while groups C and D (*p=0.566, p=0.763*) had an increase but was not significant compared to group A.

4.1 DISCUSSION

The result showed an increase in the number of entries into the open arms in groups B, C, and D compared with group A, with *p*-values of 0.762, 0.522, and 0.019, respectively. The increases observed in groups B and C were not statistically significant ($p > 0.05$), whereas the increase observed in group D was statistically significant ($p < 0.05$). The number of entries into the closed arms also increased in groups B, C, and D compared with group A; however, these increases were not statistically significant, with *p*-values of 0.574, 1.000, and 0.067, respectively.

Similarly, the time spent in the open arms increased in groups B, C, and D compared with group A, with *p*-values of 0.679, 0.396, and 0.026, respectively. The increases in groups B and C were not statistically significant, while the increase observed in group D was statistically significant. In contrast, the time spent in the closed arms decreased in groups B, C, and D compared with group A, with *p*-values of 0.341, 0.001, and 0.001, respectively. The reduction observed in group B was not statistically significant, whereas the reductions in groups C and D were statistically significant ($p < 0.05$).

Overall, the findings demonstrate a dose-related behavioural pattern, particularly at the higher doses. Group D showed significantly greater exploration and time spent in the open arms, accompanied by a significant reduction in time spent in the closed arms. This pattern of increased open-arm exploration and reduced avoidance of the open arms is consistent with an anxiolytic-like effect of the ethanolic extract of *Dioscorea bulbifera*, with the effect being most evident at the high dose.

The present study demonstrates that ethanolic extract of *Dioscorea bulbifera* produced anxiolytic-like effects in Wistar rats, particularly at the high dose. The present finding is strongly supported by the neuropharmacological study of *D. bulbifera* reported by Patel and Galani, (2017) which evaluated a hydroalcoholic tuber extract in mice. That study found that *D. bulbifera* significantly increased both the number of entries into and the time spent in the open arms of the elevated plus maze. The anxiolytic effect was also observed at specific doses and was accompanied by findings from other behavioural tests supporting central nervous system activity. This is in close agreement with the present study because both investigations demonstrate increased open-arm exploration following administration of *D. bulbifera* extract. The findings are also comparable with studies of other medicinal plants investigated using the elevated plus maze. Wijeweera *et al*. (2006) reported anxiolytic activity for *Centella asiatica* and its methanol and ethyl acetate extracts in rats. The study found that treatment increased open-arm exploration in the EPM, supporting the usefulness of medicinal plant extracts as potential sources of anxiolytic compounds. The present study is therefore consistent with these findings, particularly because both studies demonstrate that plant-derived extracts can increase behavioural indices associated with reduced anxiety.

Similarly, Caro *et al*. (2018) found that *Mentha spicata* extract significantly increased exploration and time spent in the open arms of the EPM in Wistar rats, indicating anxiolytic activity. This finding is comparable with the present result, where *D. bulbifera* increased both the number of open-arm entries and the duration spent in the open arm. The similarity suggests that the behavioural pattern produced by *D. bulbifera* is consistent with that reported for other medicinal plants with anxiolytic potential. A further comparable finding was reported by Yahaya *et al*. (2014) in a study of the aqueous stem-bark extract of *Parkia biglobosa* in Wistar rats. The study reported that lower doses of the extract (50 and 100 mg/kg) significantly increased the time spent in the open arms of the elevated plus maze, indicating anxiolytic-like activity. However, the highest dose tested (200 mg/kg) produced a different behavioural response, with increased time spent in the enclosed arm of the maze, suggesting a possible sedative or anxiogenic-like effect at the higher dose. The researchers therefore demonstrated that the behavioural effects of *P. biglobosa* varied according to dose.

The result showed a non-significant decrease in the number of trials in groups B, C, and D compared with group A, with *p*-values of 0.113, 1.000, and 0.259, respectively. This indicates that the treatment did not significantly alter the number of trials required by the animals to complete the task. In contrast, the latency period showed a statistically significant decrease in

groups B, C, and D compared with group A, with *p*-values of 0.039, 0.033, and 0.020, respectively. The reduction in latency suggests that the treated animals were able to complete the task more quickly than the control group.

Regarding the number of errors, group B showed a decrease compared with group A, although the difference was not statistically significant (p=0.684). Groups C and D showed increases in the number of errors compared with group A; however, these increases were also not statistically significant, with *p*-values of 0.566 and 0.763, respectively. Overall, the significant reduction in latency across all treatment groups, in the absence of significant changes in the number of trials and errors, suggests that the extract may have influenced task performance or learning-related behaviour without producing a significant effect on error rate. This finding is comparable with Chowdhury et al. (2025) who investigated *D. bulbifera* extract in scopolamine-induced memory impairment in ovariectomized rats. The researchers used both the elevated plus maze and Morris water maze. *D. bulbifera* extract reduced escape latency in the Morris water maze and restored time spent searching the target quadrant during the probe trial.

CONCLUSION

In conclusion, the present study demonstrates that ethanolic extract of *Dioscorea bulbifera* possesses anxiolytic-like activity in Wistar rats, with the most pronounced effect observed at the high dose. The increased open-arm exploration observed in the elevated plus maze is consistent with findings from previous studies of *D. bulbifera* and other medicinal plants, including *Centella asiatica*, *Mentha spicata*, and *Parkia biglobosa*. However, the dose-dependent variations reported across studies indicate that the behavioural effects of medicinal plant extracts may differ according to dose and plant preparation. Overall, the findings support the potential of *D. bulbifera* as a source of compounds with anxiolytic properties and provide a basis for further pharmacological investigation.

CONFLICT OF INTEREST

There was no conflict of interest.

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