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Role of Lupinus Arboreus Extract in Cannabis-Induced Psychotic Behaviors in Wistar Rats

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Abstract | **Original Research Article**

The role of *Lupinus Arboreus*, popularly known as the Nigerian Chikadoma plant extract in cannabis-induced psychotic behaviors in Wistar Rats was investigated in this study. The study was carried out in stages, starting with the collecting and identification of plant material. 25 study animals were divided into five neurobehavioural groups (1, 2, 3, 4, 5). Both extract and chemicals were administered orally for a period of 42 days. To determine all particulate parameters under inquiry, the study used established procedures. Histological and tissue biochemical studies were performed on the hippocampus. Treatment of cannabis resulted to a significantly increased ($p<0.05$) anxiety and when compared with depression but significantly impaired ($p<0.05$) memory, locomotors and exploratory assessment in the study animals which were overturned by the extract. Similarly, the treatment of the extract with *tetrahydroxyflavone* reversed the harmful effects of cannabis on the hippocampal histology of the research animals, as a result, the efficacy of the leaf extract of Nigerian Chikadoma plant may be credited to its phyto-constituents.

Keywords: *Lupinus Arboreus*, Chikadoma plant, Cannabis, psychotic behaviors.

INTRODUCTION

The Nigerian name for Lupinus (L.) arboreus is Chikadoma plant. It is a member of the Faboideae subfamily of the Fabaceae family (Papilionoideae). Fabaceae is likely the most prevalent family in dry forests and tropical rainforests, including those in Africa and America (Babatunde *et al.*, 2023).

The English name is “Yellow bush” as well-known as “Coastal bush” in USA (Northern California) and the synonym is *Lupine* (Ohadoma, 2018a).

Executive function and working memory systems supplied by the limbic system, particularly the prefrontal cortex, are frequently credited with enabling the human to adapt tactics that can change how they interact with their environment (PFC). Aging, stress, environmental pollution, and lifestyle choices are all things that could affect how normally the neural system works, which would then affect how normally the hippocampus is built. Synthetic chemicals are frequently employed to treat a variety of medical diseases, and they have the potential to change how the neural system normally functions as well (Akuodor *et al.*, 2021). Low- and middle-income nations like Nigeria turn to medicinal plants as alternative agents due to poverty in particular, along with the emergence of allergic reactions, immune suppression, and drug resistance, necessitating the creation and assessment of herbal extracts. Due to the secondary metabolites' occurrence as complexes of naturally occuring and structurally related analogues, the use of plant extracts poses less risk to human health and the environment (Ohadoma *et al.*, 2020c). In Nigeria, a plant known as Chikadoma, often referred to as Yellow bush, is used in the traditional treatment of a number of illnesses, including microbial infections, discomfort, and inflammation. There are many ethnopharmacological applications of the chikadoma plant that have drawn interest from academic study. It is used as an antinociceptive, antipyretic, antineoplastic, antileukemic, antioxidant, antiemetic, and spasmolytic treatment in a variety of folklore contexts. Chikadoma

has antimalarial, antifungal, antityphoid, antipyretic, antioxidant, and spasmolytic actions among its pharmacology and medical potential. Its antibacterial, antileukemic, antiemetic, antineoplastic, anti-inflammatory, and anti-arthritic properties are supported by other medical potentials (Ohadoma *et al*., 2019d).

Despite this plant's common use by herbalists, there are little scientific studies on its leaves' neurobehavioral effects. This justifies the ongoing enquiry.

MATERIALS & METHODS

Ethical Approval

Ethical approval was given by the Chukwuemeka Odumegwu Ojukwu University's Uli campus's Faculty of Basic Medical Science. The National Institutes of Health's recommendations for the management and care of laboratory animals were followed when handling and treating rats (NIH, 1985).

Collection and identification of plant material.

The leaves of Lupinus arboreus (Chikadoma plant) and Cannabis sativa were collected from Akwa in Anambra State and recognised in the Botany department of Nnamdi Azikiwe University in Anambra State, Nigeria.

Preparation of extract

Lupinus arboreus (Chikadoma plant) was freshly collected, cleaned in tap water, and dried for two weeks in full sunlight. The gridding apparatus was used to ground dried sample material into powder. Until it was time to utilise it, the finely ground powder was stored in sealed containers at room temperature. A clean, transparent bucket was filled with 890g of powdered material, cleaned with distilled water, and then allowed to soak in 2400ml of 0% ethanol for 24 hours at 4 OC. A stirrer was used to vigorously stir the liquid so that all of the extract could be used. The resulting mixture was quickly sieved once more using Whatman No. 1 paper and a clean, white handkerchief acting as porcelain cloth. Around 91g of a reddish-brown sticky substance were produced after completely drying the filtrate in a water bath (40OC). Prior to administration, the extract was reconstituted in 1ml of distilled water at a concentration of 1g/ml. Until use, the extract was kept chilled at 2-8 ^{OC} (Green *et al*., 2021, Ifedi *et al*., 2023, Nwafor *et al*., 201, Izunwanne *et al*., 2024, Charles *et al*., 2021, Charles *et al*., 2025)

Preparation of Cannabis

The approach from Ifedi *et al.,* (2024) was applied. Using a grinder, the dried cannabis sativa samples were mechanically ground into powder. Until it was time to utilize it, the finely ground powder was stored at room temperature in sealed containers. A clean, transparent bucket was filled with 790g of powdered material, rinsed with distilled water, then filled with 2400ml of 0% ethanol and placed in the refrigerator at 4 OC for 24 hours. To enable full extraction, the mixture was briskly agitated with a stirrer. The resulting mixture was quickly sieved through a porcelain cloth made from a clean, white handkerchief, and then through Whatman No. 1 paper. Around 84g of a dark gooey substance were produced when the filtrate was held in a beaker and dried completely in a water bath (40 OC). After that, the extract was reconstituted in 1 ml of distilled water at a concentration of 1 g/ml, and then it was administered. Prior to use, the extract was stored in a refrigerator at 2-8 OC.

Phytochemical Analysis

With slight modifications, the phytochemical analysis of the extract was done using the methods of Ifedi *et al.,* (2024), Nwafor *et al.,* (2024), Charles *et al.,* (2025). BUCK M910 Gas Chromatography with a Flame Ionization Detector was used to do the phytochemical analysis. The used column was a 15-meter RESTEK MXT-1 column (15m x 250um x 0.15um). Using a linear velocity of 30 cm/s-1 and a split-less injection of 2 ul of sample, the injector was heated to a temperature of 280 oC. Helium 5.0pa.s served as the carrier gas, flowing at a rate of 40 ml/min. The oven was preheated to 2000 degrees Fahrenheit and heated for 5 minutes at a rate of 30 degrees Fahrenheit each minute to 3300 degrees Fahrenheit. At a temperature of 3200 degrees Celsius, the detector was operational. Phytochemicals were identified by comparing the area and mass of the internal standard to the area of the newly discovered phytochemicals. In ug/g, the various phytochemical concentrations are displayed.

Lethal dose (LD_{50}) of the extract.

The extract's lethality was determined using Lorke's (1983) technique, with minor changes.
There were two steps to this method

Experimental Animal Groupings

25 rats were used for this study and were divided into groups of 5 rats each. Psychosis was induced in all the test groups by the same concentration of Cannabis 500mg/kg. The rat groupings are as follows:
Group 1: Control group (2ml/kg extract vehicle).
Group 2: Cannabis Only Group (COG 500mg/kg)
Group 3: Low extract dose group (LDEG 200mg/kg) + CNB.
Group 4: High extract dose group (HDEG 400mg/kg) + CNB
Group 5: Positive Control Group (PCG/Chlorpromazine 200mg/kg) + CNB.
Extract, chemical and drug were administered orally for a period of 42 days.

3.5 DETERMINATION OF PARTICULATE PARAMETERS DETERMINATION OF NEUROBEHAVIOURAL STUDIES

Neurobehavior Assessment

The neurobehavioral evaluation was conducted after the last administration day. Every neurobehavioral

test was timed and scored using a digital clock (Aduema *et al.*, 2017, Ogbo *et al.*, 2024, Ovie *et al*., 2021)

Elevated Plus Maze

In mouse models of CNS diseases, the Elevated plus Maze (EPM) test is used to identify neurobehavioral activity associated with anxiety. An elevated "+"-shaped labyrinth with two oppositely positioned closed arms, two oppositely positioned open arms, and a centre space make up the EPM device. The animals for this investigation were initially brought into the test room in their home cages. The plus maze was set up with the animal facing an open arm in the centre square, and it was given three to five minutes to explore the device.

Y Maze Test

The method developed by Kraeuter *et al*., (2019) and modified by Adelodun *et al*., (2021) was used to test memory. This experiment measured the ability of mice to learn and remember geographical information. The testing was done in a Y-shaped arrangement made up of three opaque wooden arms that were 120° apart from one another. The rats were placed in the middle and given full reign to explore the three arms. The Y maze's arms were identified as A, B, and C. Alcohol was used to clean the maze, and it was left to dry before being used again. Throughout each rat's time in the Y maze, the posture was held. Every rat was grabbed and put into one of the arms, which was the identical arm for every rat, facing the centre. Each rat was let loose into the Y maze's welcoming arms after 5 minutes of calm investigation.

It was noted how many times each arm (A, B, and C) was entered as well as how many spontaneous alternations occurred. The rats were placed back in their original groups once each experiment was finished.

Open Field Test

To measure locomotor and exploratory behaviours, Wistar rats used in experiments were subjected to this. To measure locomotor and exploratory activity, seven rats from each group were randomly assigned to the apparatus, a white open box (727236 cm) with black dividing lines. To avoid odour cues, both devices were cleaned with ethanol (70%) in between testing. The creatures were noted as they explored.

Lines crossed, centre square entrances, rearing, and freezing all received points. Each rat was returned to its own group after each test.

Collection of Samples

Animals were sacrificed at the Department of Physiology, Nnamdi Azikiwe University, Okofia, following the administration of the extract. The animals were first weighed before being put to sleep in their respective groups by placing them in a white bucket that was enclosed and contained a colourless substance called chloroform. A heparinized capillary tube was used to perform an ocular puncture and obtain a blood sample. The serum was extracted using a micropipette and used for the biochemical test after the blood sample was collected, placed in an EDTA container, and centrifuged for 30 minutes at 3000 rpm (Oguwike *et al*., 2021, Nwafor *et al*., 2024, Okafor *et al*., 2024, Ifedi *et al*., 2021, Ovie *et al*., 2023).

Tissues processing method (histology)

The fixative (10% formal saline) was used to fix the collected tissue and was prepared thus:
90ml of distilled water was mixed with 10ml of formalin.

Statistical Analysis of Results

Version 25 of the Statistical Packages for Social Sciences (SPSS) was used to analyse the study's data. Both post hoc LSD and ANOVA were used to analyse the brain weight data. At $p<0.05$, the data were deemed significant.

RESULTS

Table 1: P**hytochemical analysis of the leaves of Chikadoma plant.**

Phytochemicals Constituents	Abundance
Alkaloid:	++
Flavonoids:	+++
Tannins:	++
Triterenoid/Setroids:	+
Carbohydrates:	++
Saponins:	++

Key: Highly abundant = +++, Moderately present = ++, Less abundant = +, Absent = -

Table 2: Anxiety and Depression Study Using Elevated plus Maze (EPM)

Groups	Pre-psychotic EPM study		Post-psychotic EPM study	
	Open Arm/grooming (time(s) ± sem)	Closed Arm/grooming (time(s) ± sem)	Open Arm/grooming (time(s) ± sem)	Closed Arm/grooming (time(s) ± sem)
1 (CG)	09.55 ±0.12	20.33 ± 1.01	40.33 ± 1.22	120.03 ± 0.33
2 (COG)	15.07 ± 0.34 [a]	24.60 ± 0.12	37.22 ± 0.46	131.30 ± 1.02 [a]
3 (LDEG)	11.01 ± 5.01	21.53 ± 0.11	65.23 ± 4.00[b]	110.24 ± 4.12[b]
4 (HDEG)	9.73 ± 6.25[b]	17.02 ± 0.23	107.06 ±3.25[b]	170.00 ± 3.00[b]

5 (PCG)	10.00 ± 4.43	19.04 ± 0.57	90.31 ± 0.02[b]	144.05 ± 1.12[b]

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to cannabis only group.

Table 3: Memory Assessment Study Using Y Maze Test (YMT)

Groups	YMT	% diff
1 (CG)	5.02 ± 0.00	-
2 (COG)	9.57 ± 0.03[a]	-91
3 (LDEG)	7.74 ± 0.05	-54
4 (HDEG)	4.33 ±0.00[b]	14 [b]
5 (PCG)	6.55 ± 0.07	-31

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to cannabis only group.

Table 4: Locomotor and exploratory assessment using open field test (OFT)

Groups	Number of lines crossed	Rearing against the wall	Center square entries	Freezing
1 (CG)	20.02 ±1.50	09.50 ± 1.00	1.30 ± 1.20	2.03 ± 0.03
2 (COG)	13.55 ± 0.91	15.60 ± 0.10	0.90 ± 0.40	1.30 ± 1.12
3 (LDEG)	25.07 ± 0.21	11.53 ± 0.10	1.08 ± 0.02	4.24 ± 4.11[b]
4 (HDEG)	37.64 ± 0.24[b]	07.02 ± 0.20[b]	2.51 ±0.20[b]	3.00 ± 3.14
5 (PCG)	34.08 ± 0.17[b]	10.04 ± 0.50	2.11 ± 0.00[b]	4.05 ± 1.04[b]

KEY: Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to cannabis only group.

Histological Examination

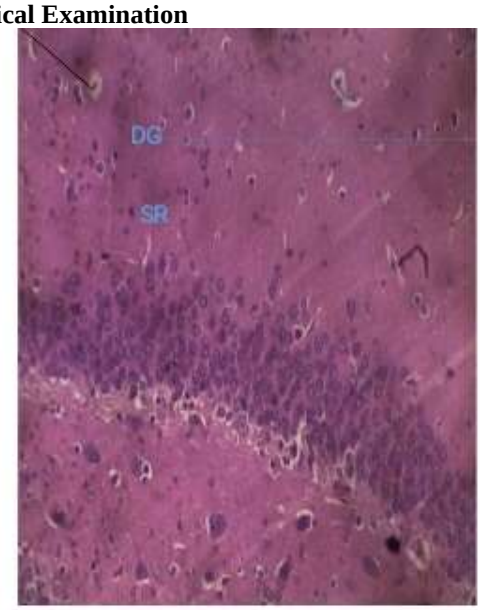

Plate1 (CG): Photomicrograph of the Hippocampus. (H&E × 400)

Well-delineated dentate gyrus (DG), with CA1 and CA2 cells in the molecular and pyramidal layers. Densely filled Stratum radiate (SR). Evidence of low level of cellular activities

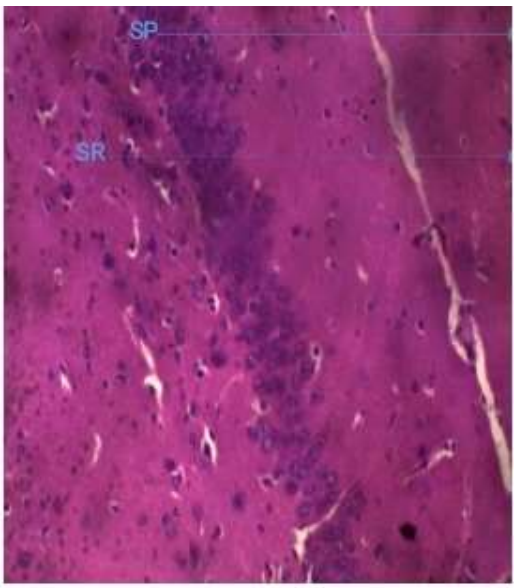

Plate2 (COG): Photomicrograph of the Hippocampus. (H&E × 400).

Clustered aggregate of cells at the stratum pyramidal (SP) – evidence of cellular assault in progress. Sparsely filled Stratum radiate (SR)

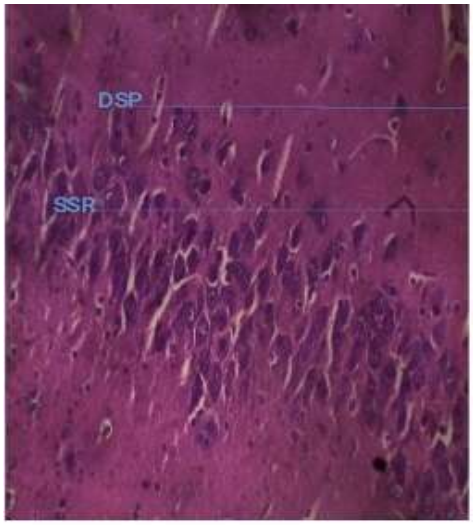

Plate3 (LDEG): Photomicrograph of the Hippocampus. (H&E × 400)

Depleted stratum pyramidal (**DSP**) cell line typifying low cellular. Scanty Stratum radiate (**SSR**) devoid of neuronal cells

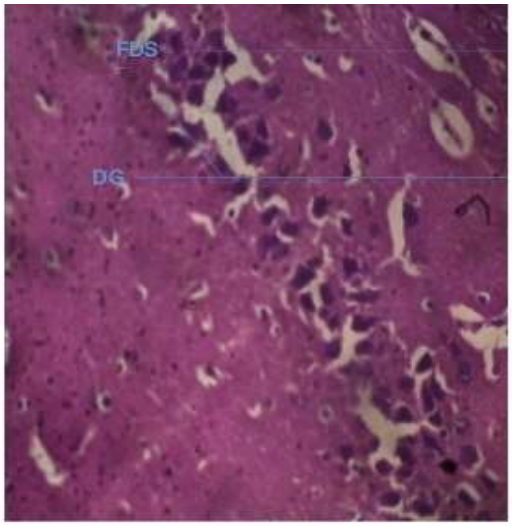

Plate4 (HDEG): Photomicrograph of the Hippocampus t (H&E × 400).

Further depletion of stratum pyramidal (**FDSP**) cell line typifying low. Visible presence of cells in the inner blade of dentate gyrus (**DG**)and deranged cellular activities in the corona radiata

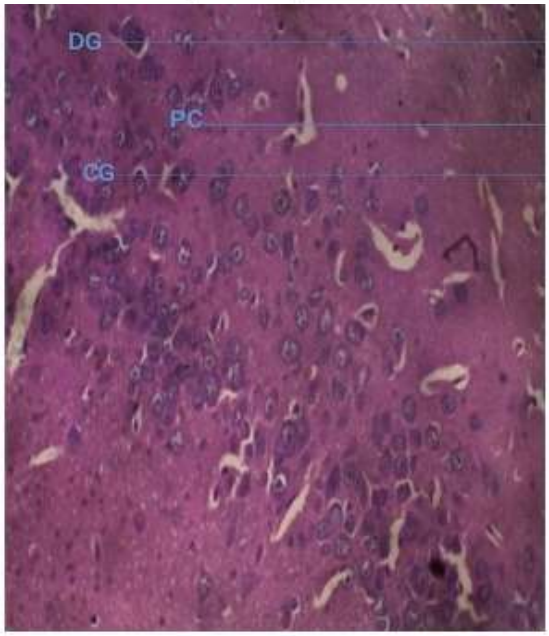

Plate5: (SDG): Photomicrograph of the Hippocampus t (H&E × 400).

Scattered CA3 cells in the dentate gyrus (**DG**)

Most pyramidal cells (**PC**) are vacuolated, disorganised and apoptotic.

Encroachment of neuronal cells (CA2) within the radiate stratum of the cingulate gyrus (**CG**)

DISCUSSION OF FINDINGS

Lorke's (1983) method was modified slightly to examine the toxicity of the ethanolic extract of Chikadoma plant leaves on wistar rats prior to treatment. The LD50 is calculated as the geometric mean of the highest non-lethal dosage and the lowest toxic dose.

There were two phases to the experiment. The first stage involves examining the hazardous spectrum. At this point, the rats were split into three groups of three each, and 150, 250, and 350 mg/kg of the extract were orally administered to the rats. Three days (72 hours) of

observation of the rats were conducted to determine their behaviour and mortality rates. Based on the indicators of intoxication and death visible in the previous stage, the dosages used in the subsequent stage were adjusted. A new batch of four rats was administered doses of 500, 1000, 1500, and 2000 mg/kg of the extract leaves, however this was because there had been no mortality in the initial stage. The treated rats were reassessed for signs of acute intoxication and mortality 72 hours later. Clinical symptoms of acute intoxication, including salivation, rhinorrhea, writhing, convulsions, tremors, ptosis, and lachrymation, behavioural changes, including spontaneous movement in the cage, climbing, and face cleaning, stress-related symptoms, including erection of fur and exophtalmia, and animal mortality rates were all absent at the conclusion of the experiment at both stages. The study's findings imply that the extract is apparently safe to use because it does not appear to cause physical, behavioural, or physiological death even at levels of 2000 mg/kg.

Additionally evaluated in this study were the Wistar rats' hippocampal cytoarchitecture, learning, memory, and locomotor functions after toxicity caused by cannabis. Cannabis sativa, one of the first plants with both therapeutic and recreational uses, is abused often due to its high concentration of the psychoactive substance THC. One of the cortical regions undergoing phylogenetic and ontogenetic development is the prefrontal cortex, which is also home to the hippocampus region, which is abundant in neurotransmitter systems like the dopamine and cholinergic systems. They are crucial in the regulation of different executive brain processes through its diverse areas and have reciprocal and extensive connections with a variety of subcortical structures. F&R2022, Friedman & Robbins. The function of Cannabis sativa has been the subject of numerous contradicting publications to date (Pratt *et al*., 2019). While some users have noted positive effects, it is well known that Cannabis sativa can also have negative effects. As a result, it is important to investigate how the Chikadoma plant may affect cognitive abilities such as learning and memory after cannabis toxicity exposure. According to the neurobehavioral tests, group B (COG) significantly decreased compared to group A (CG) in terms of freezing, centre square entries, and the number of lines crossed. However, when test groups C (LDEG), D (HDEG), and E (PCG) were compared to group B (COG), the administration of Chikadoma plant extract revealed that there was a significant increase in freezing, centre square entries, and the number of lines crossed.

Rearing frequency in group B (COG) was substantially higher than in group A (CG), but significantly lower than in group B (COG) in the test groups (C-LDEG, D-HDEG, and E-PCG). The decrease in the number of lines crossed in the cannabis-only group (COG) and the rise in the frequency of rearing indicate, respectively, that cannabis may have an impact on locomotor activity and the initiation of anxiety-like behaviours. The injection of Chikadoma plant extract, however, restored the rats' deficiencies in locomotion and anxiety-like behaviours.

Additionally, the elevated plus maze study on anxiety and depression revealed that the post-psychotic groups' rats spent significantly different amounts of time in the open and closed arms of the elevated plus maze. When compared to the control group, it was found that the rats in all post-psychotic groups spent more time in the open and closed arms of the elevated plus maze. This is a result of cannabis-induced despair and anxiety in the rats.

One article that claimed cannabis can cause psychotic symptoms at large doses supports this conclusion. Cannabis addicts are more prone to experience psychosis, according to a related study (Kasten *et al*., 2019, Imam *et al*., 2017). However, it has been found that the amount of time spent in the open and closed arms of the post-psychotic elevated plus maze study increased more after the extract was administered in low and high doses, as well as in the PCG standard drug group. These findings are corroborated by reports that a range of movement problems can be brought on by antipsychotic medications. Antipsychotic medications have also been linked to a variety of movement problems, some of which can be distressing and untreatable (Haddad & Correll 2018). The extract employed in this investigation may be superior than PCGs and have a strong antipsychotic potential.

Rats exposed to Cannabis sativa and Chikadoma plant extract underwent the Y-Maze test to measure cognition, spatial learning, and memory. While there was a discernible decline in all the test groups when compared with the cannabis only group (COG), there was a significant increase in the total arm entries and percentage difference of the cannabis only group (COG) when compared to the control group. The current findings are consistent with a prior study (Enogieru & Omoruyi 2022) in which a plant extract corrected cognitive impairments in rats.

The results of the histological examination, as displayed in the photomicrograph of plate 1 (group A-CG), demonstrated that the hippocampus had a normal histoarchitecture. It has been noted that the dentate gyrus is clearly defined and contains cornuammonis cells as well as cells in the molecular, pyramidal, and multiform layers. It has been noted that stratum radiata are densely filled, which is proof of regular cellular activity. The layers and thickness of the cortex were observed to be normal. The photomicrograph of plate 2 (group B - COG) shows that the number of cells at the stratum pyramidal has significantly decreased and has aggregated into clusters. This is a sign of ongoing cellular damage to the hippocampus' histoarchitecture. The stratum radiata's cellular population was found to be

extremely sparse, and this was accompanied by poorly defined cellular nuclei and cellular outlines.

These results corroborated those of Owoeye *et al*. (2019), who reported that plant extracts had a protective effect on hippocampal damage. It has been suggested that the main psychoactive ingredient in cannabis, delta-9-tetrahydrocannabinol (THC), interacts with the hippocampus' cannabinoid type 1 (CBI) receptor to cause cell shrinkage. It was noted from the photomicrograph of plate 3 (group C - LDEG) that the stratum pyramidal demarcation is slightly diminished, indicating abysmally low cellular activity. As a result, there was significant cellular proliferation and regeneration in the pyramidal cell layer. In the photomicrograph of plates 4 (group D-HDEG) and 5 (group E-PCG), the histoarchitecture of the hippocampus was significantly improved. In the pyramidal cell layer, the majority of the pyramidal cells are seen to be multiplying. This result is consistent with a report suggesting that the extract's anti-depressant properties may be due to its phyto-constituents (Alagan *et al*., 2019, Agbon *et al*., 2014). Although the precise mechanism by which Cannabis sativa causes neuronal degeneration, locomotor dysfunction, and cognitive deficits is not fully understood, it is possible that its metabolite contributes to the production of ROS. (Wolff *et al*., 2015; Omeiza *et al*., 2021) THC from Cannabis sativa has been shown to cause brain mitochondrial respiratory chain malfunction and increase oxidative stress. According to the phytochemical analyses conducted for this study, the Nigerian Chikadoma plant's leaf extract included high concentrations of alkaloids, glycosides, steroids, saponins, terpenes, and flavonoids. This high concentration of phytochemicals may be the cause of the plant's neurological and hematopoietic effects, which are consistent with claims that plants strong in phytoconstituents have brain- and blood-boosting characteristics. The findings in this study could be explained by the abundance of phytochemicals, including flavonoids, a highly effective antioxidant that also suggests the significance of the plant in folklore (Charles *et al*., 2022; Enogieru *et al*., 2021; Anyanwu *et al*., 2020).

Disclaimer (Artificial Intelligence)

The authors hereby declare that no generative AI technologies, such as text-to-image generators or large language models (ChatGPT, COPILOT, etc.), were used in the writing or editing of this manuscript.

Consent: It doesn't apply.

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