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# Effects of VIP AK-47 Akuskura on Oral Microbial Flora, Antimicrobial Activity, and Histopathology of Motor Neuron-Related Brain Regions in Mice

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**Abstract**

VIP AK-47 Akuskura is an emerging new psychoactive substance (NPS) increasingly abused among youths in Northern Nigeria, particularly through gargling and oral exposure. Despite its widespread use, little is known regarding its chemical composition, biological effects, or mechanisms of action. This study investigated the effects of VIP AK-47 Akuskura on oral microbial flora, antimicrobial activity, motor coordination, and histopathology of motor neuron-related brain regions in mice.Twenty-four adult male albino mice were randomly divided into four groups: control, low-dose (600mg/kg), medium-dose (800mg/kg), and high-dose (1000mg/kg). The substance was administered through gargling for two weeks. Oral microbial analysis, antimicrobial susceptibility testing, open field test, beam walk test, footprint analysis, and histopathological examination of the cerebral cortex, basal ganglia, and cerebellum were conducted.The findings revealed dose-dependent increases in body weight among treated groups. Significant alterations in oral microbial flora were observed, characterized by reductions in beneficial commensal microorganisms and increases in opportunistic organisms such as *Staphylococcus aureus* and *Candida albicans*. VIP AK-47 Akuskura demonstrated moderate antimicrobial activity against selected oral microorganisms, particularly Gram-positive bacteria.Neurobehavioral assessment using the open field test showed no significant changes in general locomotor activity. However, beam walk and footprint analyses demonstrated impaired motor coordination, increased hind foot slips, prolonged beam crossing time, and altered gait patterns in medium- and high-dose groups. Histopathological examination revealed neuronal degeneration, vacuolation, pyknosis, chromatolysis, eosinophilia, Purkinje cell degeneration, and necrosis within the cerebral cortex, basal ganglia, and cerebellum.The study concluded that VIP AK-47 Akuskura exerts dose-dependent neurotoxic effects, alters oral microbial ecology, and impairs motor coordination in mice. The observed histopathological alterations suggest significant neuronal injury involving motor coordination pathways. These findings highlight potential public health risks associated with the abuse of VIP AK-47 Akuskura and emphasize the urgent need for chemical characterization, toxicological evaluation, regulatory surveillance, and public health awareness regarding emerging psychoactive substances in Nigeria.

Keywords
VIP AK-47,
Akuskura,
Oral microbial flora,
Antimicrobial activity,
Histopathology,
Basal ganglia,
Cerebellum,
Mice,
Neurotoxicity.

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**Introduction**

The rapid rise in the use of New Psychoactive Substances (NPSs) has become a major global public health concern. NPSs are synthetic or herbal psychoactive compounds designed to mimic the effects of conventional illicit drugs while evading legal regulation. According to the United Nations Office on Drugs and Crime (UNODC), NPSs are substances of abuse, in pure form or preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, yet pose significant public health threats (UNODC, 2013). These substances are associated with severe neurological, cardiovascular, psychiatric, and behavioural disturbances, including seizures, agitation, aggression, acute psychosis, and potential dependence (Luethi and Liechti, 2020).

In Northern Nigeria, a novel psychoactive preparation known as VIP AK-47 Akuskura (also called Kuskura or Akurkura) has gained widespread popularity, particularly among youths and commercial workers. Derived from the Hausa word meaning "gargling" or "rinsing," Akuskura is commonly administered through oral rinsing or gargling. Users believe it relieves catarrh, malaria, headache, body pains, fatigue, and enhances alertness and sexual arousal. The preparation typically consists of herbal mixtures laced with tobacco, cannabis derivatives, and other unidentified psychoactive compounds (Mahmud, 2022; Tijjani et al., 2025).

Previous preclinical investigations demonstrated that VIP AK-47 Akuskura induces motor coordination impairment and histopathological alterations in the cerebral cortex, basal ganglia, and cerebellum of mice following repeated gargling administration (Your Previous Study, 2025).

The oral cavity harbours a complex and diverse microbial ecosystem composed of bacteria, fungi, and viruses, collectively known as the oral microbiota. This community plays a crucial role in maintaining oral health by preventing pathogen colonisation, modulating immune responses, and supporting systemic wellbeing. Dysbiosis of the oral microbiota has been linked to oral infections, periodontal disease, halitosis, and increased risk of systemic complications (Dewhirst et al., 2010; Lamont et al., 2018).

Substances administered via gargling come into prolonged direct contact with the oral mucosa and microbial flora. Tobacco and cannabis-containing preparations have been shown to significantly alter oral bacterial composition, often exhibiting selective antimicrobial activity while promoting dysbiosis and enrichment of specific pathobionts (Kumar et al., 2015; Newland et al., 2021). While moderate antimicrobial effects may offer perceived therapeutic benefits (e.g., against catarrh), excessive or prolonged disruption of beneficial microbes can predispose users to opportunistic infections and long-term oral health deterioration.

Motor neuron-related brain regions, including the motor cortex (upper motor neurons), basal ganglia, cerebellum, and brainstem nuclei, are critical for motor planning, coordination, gait, balance, posture, and motor learning (Bostan and Strick, 2018; Yoshida et al., 2022). Neurotoxic insults to these areas often manifest as histopathological changes such as neuronal vacuolation, pyknosis, chromatolysis, Nissl substance dispersal, and gliosis, leading to motor deficits (Ibegbu et al., 2014; Ajibade et al., 2006).

Despite the increasing abuse of VIP AK-47 Akuskura across Nigeria and parts of West Africa, there remains limited scientific information on its effects on the oral microbial ecology, its antimicrobial properties against oral pathogens, and its specific neuro-histopathological impact on motor neuron-related brain regions. Therefore, this study aimed to investigate the effects of VIP AK-47 Akuskura on oral microbial flora, its antimicrobial activity, and the histopathology of motor neuron-associated brain tissues in mice.

## Materials and Methods

**Study Area**

The research was conducted in the Department of Human Physiology and the Medical Microbiology Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University, Kano, Nigeria. Kano is the capital of Kano State, located in Northern Nigeria at coordinates 11.7470° N, 8.5247° E. It is bounded by Jigawa State to the Northeast, Bauchi to the Southeast, Kaduna to the Southwest, and Katsina to the Northwest.

**Experimental Animals** A total of twenty-four (24) healthy adult male albino mice, aged 7–8 weeks and weighing 18–25 g, were used for the study. The animals were housed in clean plastic cages (maximum of five mice per cage) under standard laboratory conditions: 12-hour light/dark cycle, temperature of 22 ± 2°C, relative humidity of 50–60%, and good ventilation. They had free access to standard rodent chow and clean water *ad libitum*. The mice were acclimatized for two weeks prior to the commencement of the experiment.

**Preparation and Administration of VIP AK-47 Akuskura** VIP AK-47 Akuskura was obtained from the National Drug Law Enforcement Agency (NDLEA), Kano State Command, Nigeria. Authentication and preliminary phytochemical analysis were performed at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University, Kano, using the method of Evans and Trease (2002). The extract was prepared in distilled water at concentrations corresponding to low (600 mg/kg), medium (800 mg/kg), and high (1000 mg/kg) doses. Administration was done via oral gargling using sterile cotton swabs soaked in the respective extract concentrations on alternate days for two weeks. The control group received 0.3 mL of distilled water only.

**Experimental Design** The mice were randomly divided into four groups (n = 6 per group):

- **Group I (Control)**: Received distilled water (0.3 mL) via gargling.
- **Group II (Low dose)**: Received 600 mg/kg body weight of VIP AK-47 Akuskura extract.
- **Group III (Medium dose)**: Received 800 mg/kg body weight of VIP AK-47 Akuskura extract.
- **Group IV (High dose)**: Received 1000 mg/kg body weight of VIP AK-47 Akuskura extract.

All treatments were administered on alternate days for a period of two weeks.

**Collection and Analysis of Oral Swabs** Sterile cotton swabs were used to collect oral samples from each mouse before the first administration and 24 hours after the last dose on day 14. The swabs were immediately transported to the microbiology laboratory and inoculated onto Nutrient agar, Blood agar,

MacConkey agar, and Sabouraud Dextrose agar. Plates were incubated at 37°C for 24–48 hours (bacteria) and 25°C for 72 hours (fungi). Microbial isolates were identified using standard microbiological techniques including Gram staining, colony morphology, catalase test, coagulase test, and sugar fermentation tests.

**Antimicrobial Activity of the Extract** The antimicrobial activity of VIP AK-47 Akuskura extract was evaluated using the agar well diffusion method against selected oral microorganisms: *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans*. Standardized inocula (0.5 McFarland standard) were spread on Mueller-Hinton agar plates. Wells (6 mm diameter) were punched and filled with 100 µL of different concentrations of the extract. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined where applicable.

**Assessment of Motor Coordination** Motor coordination was evaluated using the beam walk test and footprint analysis.

**Beam Walk Test** The apparatus consisted of a 1-meter-long wooden beam with a 12 mm wide flat surface, elevated 50 cm above the ground. Mice were trained to traverse the beam prior to the experiment. On the test day, each mouse was placed at one end of the beam immediately after the last treatment, and the time taken to cross 80 cm of the beam as well as the number of foot slips were recorded.

**Footprint Analysis** Footprint analysis was performed according to the protocol of Sugimoto and Kawakami (2019). Forepaws and hind paws of mice were painted with different non-toxic dyes. Mice were allowed to walk along a narrow paper-covered runway toward an enclosed goal box. Stride length (forelimb and hind limb), overlap, and base width were measured from the dried footprints.

**Histopathological Analysis** At the end of the experiment, mice were humanely sacrificed under anesthesia. Brains were carefully removed and fixed in 10% neutral buffered formalin for 48 hours. Specific regions of interest — motor cortex (Layer V), basal ganglia, cerebellum, and brainstem motor nuclei — were dissected, processed through graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a rotary microtome and stained with Hematoxylin and Eosin (H&E). Slides were examined under a light microscope for histopathological changes such as neuronal vacuolation, pyknosis, chromatolysis, Nissl substance dispersal, and gliosis, with particular attention to motor neurons.

**Statistical Analysis** All data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using SPSS version 25. One-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test was used to compare differences between groups. Paired t-test was used where applicable (e.g., pre- and post-treatment comparisons). A value of $P < 0.05$ was considered statistically significant.

**Ethical Consideration** The study was approved by the Animal Care and Use in Research Ethics Committee (ACUREC) of Bayero University, Kano (Approval No: BUK/ACUREC/CAP/PG3). All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

## Results

### Assessment of Oral Microbial Flora Following Two Weeks of Gargling with VIP AK-47 Akuskura

**Table 1: Comparison of Oral Microbial Flora Among Experimental Groups Before and After Exposure to VIP AK-47 Akuskura**

| Parameters | Group 1: Control (n=6) Mean±SEM | Group 2: Low Dose (600mg/kg) Mean±SEM | Group 3: Medium Dose (800mg/kg) Mean±SEM | Group 4: High Dose (1000mg/kg) Mean±SEM | P-Value |
|---|---|---|---|---|---|
| Streptococcus spp. colony count (CFU/ml) | 5.83±0.47 | 4.67±0.33 | 3.17±0.48a | 2.83±0.40a | 0.012 |
| Staphylococcus aureus colony count (CFU/ml) | 1.17±0.30 | 2.33±0.42 | 3.50±0.56a | 4.17±0.47a | 0.008 |
| Candida albicans colony count (CFU/ml) | 0.67±0.21 | 1.50±0.22 | 2.67±0.33a | 3.33±0.42a | 0.005 |
| Lactobacillus spp. colony count (CFU/ml) | 4.50±0.34 | 3.83±0.31 | 2.67±0.21a | 2.17±0.30a | 0.010 |

One-way ANOVA; SEM = Standard Error of Mean; * indicates statistical significance; Tukey post hoc test; 'a' indicates statistical significance compared with control group ($P<0.05$).

Table 1 summarizes the effects of VIP AK-47 Akuskura on oral microbial flora after two weeks of gargling. Statistical analysis demonstrated significant dose-dependent reductions in beneficial oral commensals such as *Streptococcus* spp. and *Lactobacillus* spp. in medium- and high-dose groups compared to controls. Conversely, there were dose-dependent increases in opportunistic organisms including *Staphylococcus aureus* and *Candida albicans* in treated groups.

The reduction in normal oral flora may suggest that VIP AK-47 Akuskura possesses antimicrobial constituents capable of altering oral microbial homeostasis. Similar alterations in oral microbial composition have been associated with exposure to tobacco-containing psychoactive substances and cannabinoid-related products. The observed increase in opportunistic microorganisms could predispose users to oral infections, mucosal irritation, and periodontal abnormalities.

**Assessment of Antimicrobial Activity of VIP AK-47 Akuskura**

**Table 2: Antimicrobial Activity of VIP AK-47 Akuskura Against Selected Oral Microorganisms**

| Microorganisms | Low Dose (600mg/kg) Zone of Inhibition (mm) | Medium Dose (800mg/kg) Zone of Inhibition (mm) | High Dose (1000mg/kg) Zone of Inhibition (mm) | P-Value |
|---|---|---|---|---|
| *Staphylococcus aureus* | 10.50±0.56 | 13.67±0.49a | 15.83±0.60a | 0.003 |
| *Streptococcus mutans* | 9.17±0.40 | 11.83±0.48 | 13.50±0.42a | 0.008 |
| *Escherichia coli* | 6.33±0.33 | 8.17±0.40 | 9.50±0.34 | 0.071 |
| *Candida albicans* | 8.50±0.43 | 10.33±0.56 | 11.83±0.47a | 0.015 |

One-way ANOVA (mm); * indicates statistical significance; Tukey post Hoc Test; 'a' indicates significant difference compared with low-dose group (P<0.05).

Table 2 shows the antimicrobial activity of VIP AK-47 Akuskura against selected oral microorganisms. The extract demonstrated dose-dependent antimicrobial activity, particularly against Gram-positive organisms such as *Staphylococcus aureus* and *Streptococcus mutans*. Significant increases in zones of inhibition were observed in medium- and high-dose groups.

The antimicrobial activity observed may be attributed to phytochemical constituents present in the substance, including alkaloids, flavonoids, nicotine-related compounds, or cannabinoid derivatives. However, despite the similarities between VIP AK-47 Akuskura and other new psychoactive substances (NPSs) in terms of psychoactive effects, the actual chemical composition of the substance remains unknown; therefore, direct comparison with other NPSs remains limited.

The relatively lower antimicrobial activity against *Escherichia coli* may be due to the resistance conferred by the outer membrane of Gram-negative bacteria, which reduces permeability of antimicrobial agents.

**Assessment of Motor Coordination Parameters Using Beam Walk Test**

**Table 3: Comparison of Neurobehavioral Parameters in Beam Walk Test Between Control and Treatment Groups**

| Parameters | Group 1 (Control) | Group 2 (Low Dose) | Group 3 (Medium Dose) | Group 4 (High Dose) | P-Value |
|---|---|---|---|---|---|
| Time spent to cross beam (sec) | 8.50±0.52a | 15.67±2.41 | 34.83±8.50a | 19.50±3.33 | 0.021 |
| Frequency of right hind foot slips | 0.00±0.00ab | 1.67±0.42 | 3.00±0.57a | 2.83±0.47b | 0.013 |

| Parameters | Group 1 (Control) | Group 2 (Low Dose) | Group 3 (Medium Dose) | Group 4 (High Dose) | P-Value |
|---|---|---|---|---|---|
| Frequency of left hind foot slips | 0.00±0.00 | 0.83±0.30 | 1.50±0.42 | 1.33±0.33 | 0.201 |

One-way ANOVA; * indicates statistical significance; Tukey post Hoc Test; 'a' indicates statistical significance between groups 1 and 3 ($P<0.05$); 'b' indicates significance between groups 1 and 4 ($P<0.05$).

Table 3 summarizes the effects of VIP AK-47 Akuskura on motor coordination using beam walk analysis. Following two weeks of gargling, mice treated with medium- and high-doses demonstrated increased beam crossing time and increased frequency of hind foot slips compared with control group.

The increased beam crossing time observed in the medium-dose group suggested impaired motor coordination and balance. The increase in right hind foot slips further suggested that VIP AK-47 Akuskura may adversely affect motor pathways associated with balance and neuromuscular coordination.

The beam walk test is commonly used to assess cerebellar and basal ganglia functions in rodents. Deficits observed in this study may indicate neurotoxicity involving motor neuron-related brain regions. Similar findings have been reported in studies involving psychoactive substances and cannabinoid exposure, where locomotor impairment, muscular rigidity, and gait abnormalities were observed.

Despite these similarities, the precise mechanism of action of VIP AK-47 Akuskura remains unknown because its exact chemical constituents have not yet been characterized.

**Assessment of Motor Coordination Parameters Using Footprint Analysis**

**Table 4: Comparison of Motor Activity from Footprint Analysis Test in Control and Treatment Groups**

| Parameters | Group 1 (Control) | Group 2 (Low Dose) | Group 3 (Medium Dose) | Group 4 (High Dose) | P-Value |
|---|---|---|---|---|---|
| Stride length of forelimb (cm) | 4.37±0.22ab | 5.30±0.34 | 5.97±0.28a | 5.73±0.24b | 0.005 |
| Stride length of hind limb (cm) | 4.40±0.25a | 5.27±0.30 | 5.93±0.26a | 5.60±0.29 | 0.011 |
| Overlap | 0.33±0.06 | 0.40±0.07 | 0.47±0.08 | 0.70±0.20 | 0.173 |
| Forelimb base width (cm) | 1.80±0.13 | 1.67±0.11 | 1.60±0.12 | 1.57±0.16 | 0.701 |
| Hind limb base width (cm) | 2.57±0.16 | 2.53±0.14 | 2.37±0.11 | 2.50±0.22 | 0.904 |

One-way ANOVA; * indicates statistical significance; Tukey post Hoc Test.

Table 4 shows the footprint analysis following exposure to VIP AK-47 Akuskura. Significant increases in forelimb and hind limb stride lengths were observed in medium- and high-dose groups compared to control group.

The increased stride lengths suggested alterations in locomotor coordination and gait patterns. Although increased stride length may sometimes indicate improved locomotor activity, in this context it may reflect compensatory gait adaptation secondary to impaired motor coordination.

There were no statistically significant changes in overlap, forelimb base width, or hind limb base width among experimental groups, indicating that VIP AK-47 Akuskura had limited effects on limb stability parameters.

Overall, the findings suggested that medium- and high-doses of VIP AK-47 Akuskura altered gait patterns and motor coordination in mice in a dose-dependent manner.

**Assessment of Body Weight**

**Table 5: Comparison of Initial and Final Body Weights Among Experimental Groups**

| Parameters | Group 1: Control (n=6) Mean±SEM | Group 2: Low Dose (600mg/kg) Mean±SEM | Group 3: Medium Dose (800mg/kg) Mean±SEM | Group 4: High Dose (1000mg/kg) Mean±SEM | P-Value |
|---|---|---|---|---|---|
| Initial Weight (g) | 20.42±0.31 | 20.73±0.37 | 21.95±1.02 | 20.68±0.28 | 0.584 |
| Final Weight (g) | 22.63±0.29a | 24.52±0.96 | 26.71±0.41a | 26.48±1.21 | 0.041 |
| Percentage Body Weight Gain (%) | 19.84±4.02 | 13.52±6.48 | 28.71±5.07 | 36.84±8.91 | 0.063 |

**Histology of the Cerebral Cortex After Two Weeks of Gargling with VIP AK-47 Akuskura**

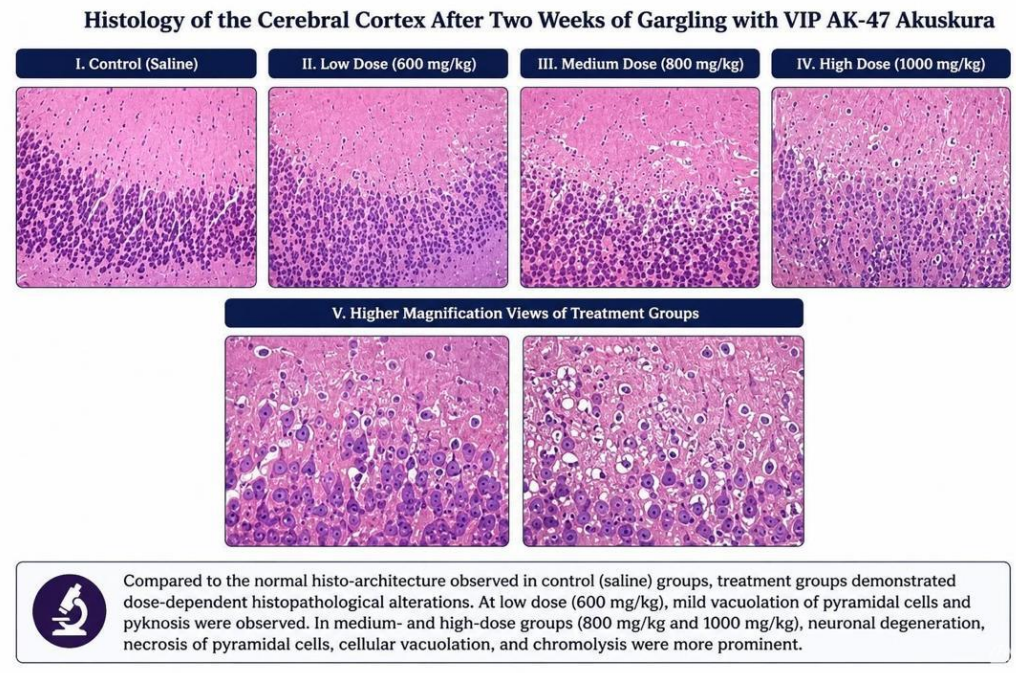


Plate 1: Micrographs of the Cerebral Cortex of Control and Treatment Groups

Plate 1 shows the photomicrographs of the cerebral cortex following two weeks of gargling with VIP AK-47 Akuskura. Compared to the normal histo-architecture observed in

control groups, treatment groups demonstrated dose-dependent histopathological alterations.

At low dose (600mg/kg), mild vacuolation of pyramidal cells and pyknosis were observed. In medium- and high-dose groups (800mg/kg and 1000mg/kg), neuronal degeneration, necrosis of pyramidal cells, cellular vacuolation, and chromatolysis were more prominent.

These findings suggested that VIP AK-47 Akuskura may induce cellular stress, neuronal injury, and neurotoxicity within the cerebral cortex. Pyramidal neurons constitute major excitatory neurons in the cerebral cortex and are essential for information processing, memory, and motor integration. Alterations in pyramidal cell morphology may therefore affect cortical neuronal function.

The vacuolations observed in this study may represent reversible cellular injury, whereas pyknosis indicates irreversible neuronal degeneration associated with apoptosis or necrosis.

**Histology of the Basal Ganglia After Two Weeks of Gargling with VIP AK-47 Akuskura**

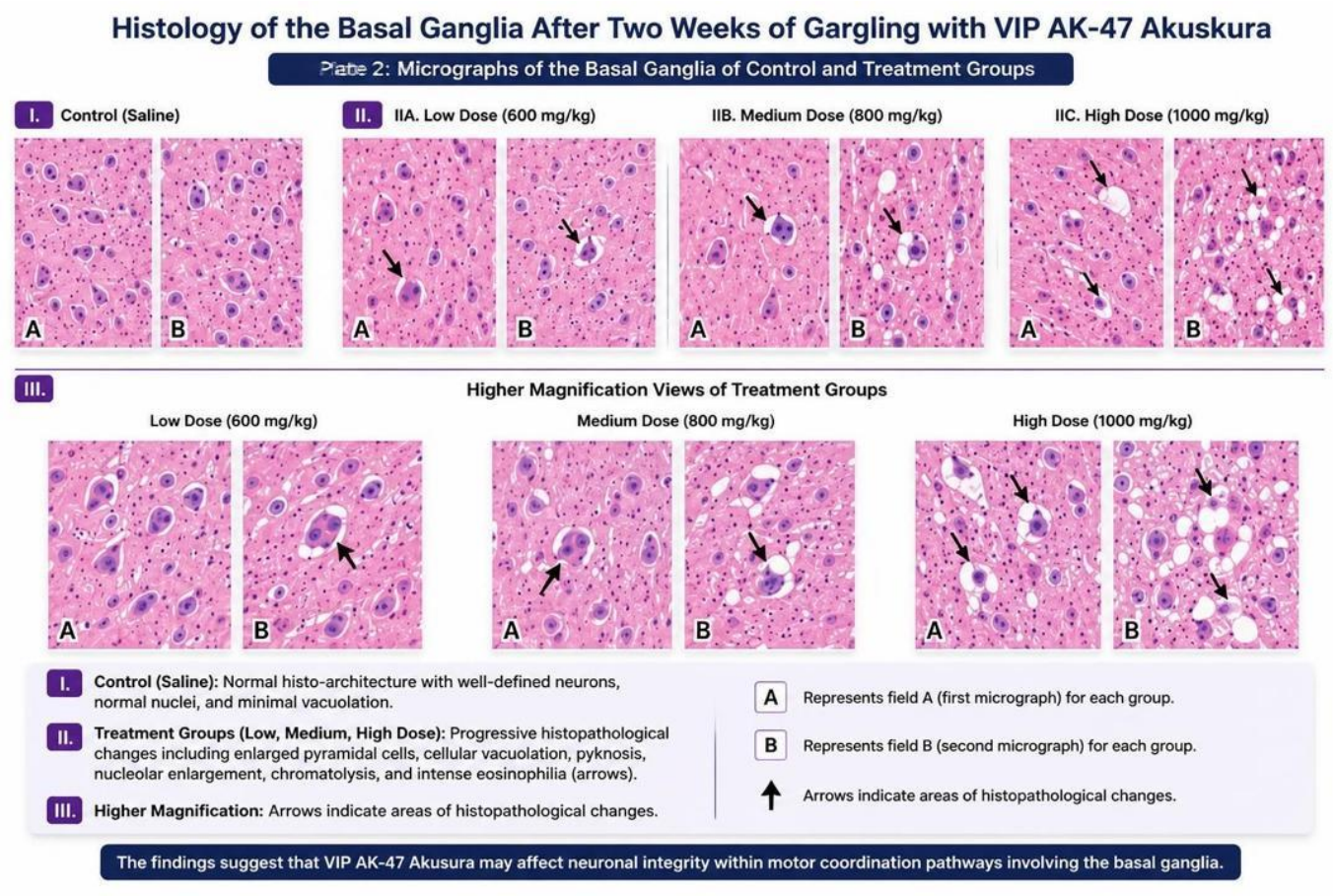


Plate 2: Micrographs of the Basal Ganglia of Control and Treatment Groups

Plate 2 demonstrates the photomicrographs of the basal ganglia following exposure to VIP AK-47 Akuskura. Compared to normal histo-architecture of the control group, treatment groups exhibited enlarged pyramidal cells, cellular vacuolation, pyknosis, nucleolar enlargement, chromatolysis, and intense eosinophilia.

The observed dispersal of Nissl substances from the center toward the periphery of neurons suggested chromatolytic changes associated with neuronal stress or injury. Enlargement of nucleoli may indicate increased cellular metabolic stress responses.

The intense eosinophilia observed may suggest tissue injury and inflammatory reactions within the basal ganglia. Similar histological alterations have been associated with neurotoxicity induced by psychoactive substances and other neuro-degenerative agents.

The findings further suggested that VIP AK-47 Akuskura may affect neuronal integrity within motor coordination pathways involving the basal ganglia.

**Histology of the Cerebellum After Two Weeks of Gargling with VIP AK-47 Akuskura**

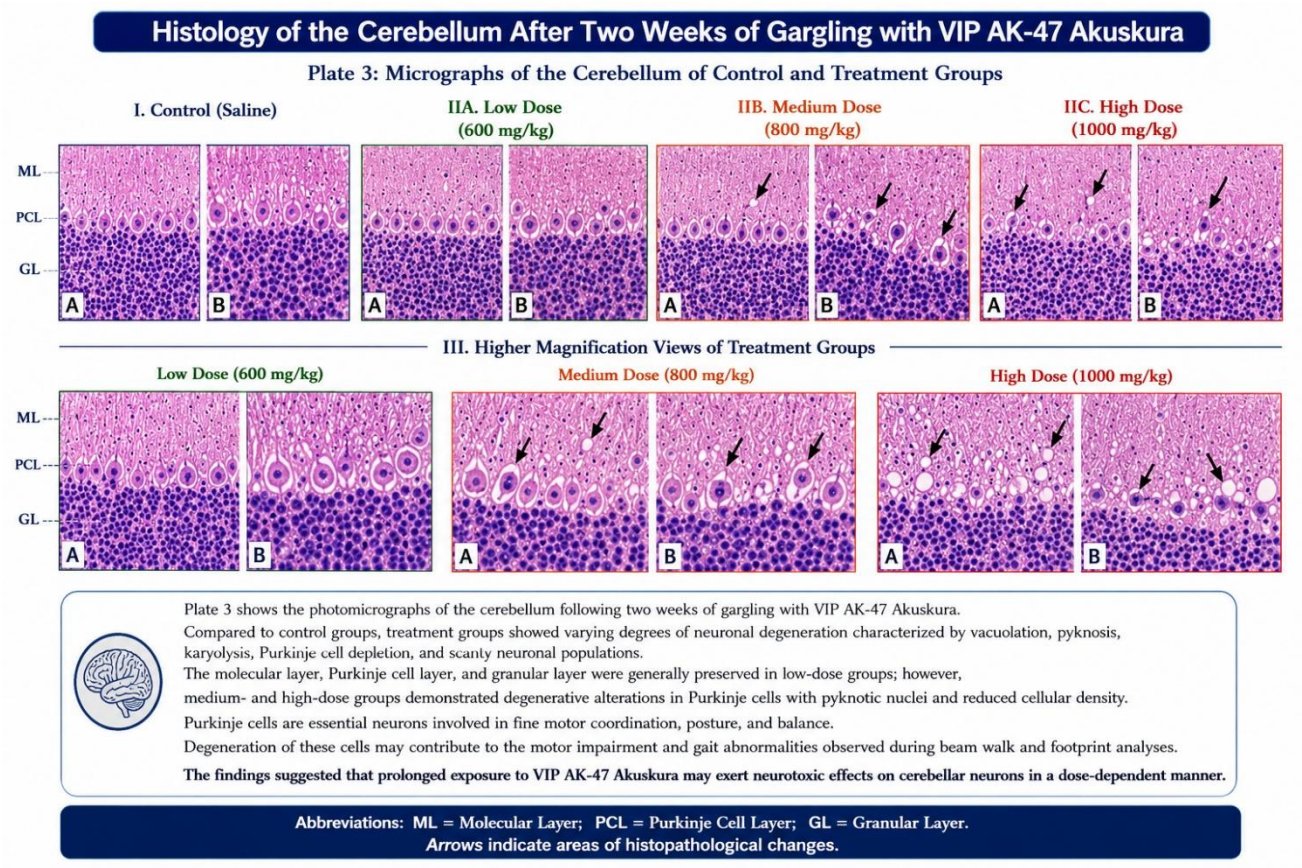


Plate 3: Micrographs of the Cerebellum of Control and Treatment Groups

Plate 3 shows the photomicrographs of the cerebellum following two weeks of gargling with VIP AK-47 Akuskura. Compared to control groups, treatment groups showed varying degrees of neuronal degeneration characterized by vacuolation, pyknosis, karyolysis, Purkinje cell depletion, and scanty neuronal populations.

The molecular layer, Purkinje cell layer, and granular layer were generally preserved in low-dose groups; however, medium- and high-dose groups demonstrated degenerative alterations in Purkinje cells with pyknotic nuclei and reduced cellular density.

Purkinje cells are essential neurons involved in fine motor coordination, posture, and balance. Degeneration of these cells may contribute to the motor impairment and gait abnormalities observed during beam walk and footprint analyses.

The findings suggested that prolonged exposure to VIP AK-47 Akuskura may exert neurotoxic effects on cerebellar neurons in a dose-dependent manner.

## Discussion

The present study investigated the effects of VIP AK-47 Akuskura on oral microbial flora, antimicrobial activity, motor coordination, gait patterns, and histopathological alterations in mice following two weeks of gargling exposure. Although VIP AK-47 Akuskura appears to share psychoactive similarities with other new psychoactive substances (NPSs), its actual chemical composition and mechanisms of action remain unknown. Therefore, comparisons with other psychoactive substances were made cautiously and mainly based on observed behavioural and histological similarities (Peacock et al., 2019; Zawilska & Wojcieszak, 2021).

The assessment of body weight revealed dose-dependent increases in body weight gain among treated mice, particularly in the medium- and high-dose groups. This observation suggested that VIP AK-47 Akuskura may influence appetite regulation and metabolic processes. Similar increases in body weight have been reported following exposure to psychoactive substances containing cannabinoids and tobacco-related compounds, which are known to stimulate feeding behaviour and alter energy metabolism (Le Foll et al., 2013).

Cannabinoid-related substances may activate central reward pathways and hypothalamic feeding centers, resulting in increased food intake and weight gain. The observed increase in body weight in this study may therefore indicate metabolic effects associated with prolonged exposure to VIP AK-47 Akuskura.

Assessment of oral microbial flora demonstrated significant reductions in beneficial oral commensals such as *Streptococcus* spp. and *Lactobacillus* spp., with concurrent increases in opportunistic organisms including *Staphylococcus aureus* and *Candida albicans*. This suggested that VIP AK-47 Akuskura altered oral microbial homeostasis. The antimicrobial properties of the substance may have contributed to suppression of normal oral flora, thereby creating an environment favourable for opportunistic microorganisms. Alteration of oral microbial balance is clinically important because disruption of normal oral flora may predispose individuals to oral infections, periodontal disease, mucosal inflammation, and halitosis (Dewhirst et al., 2010).

The antimicrobial susceptibility study further demonstrated that VIP AK-47 Akuskura exhibited moderate antimicrobial activity against selected oral microorganisms, particularly Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus mutans*. The antimicrobial effects observed may be associated with the presence of phytochemicals such as alkaloids, flavonoids, nicotine-like compounds, or cannabinoid derivatives. Similar antimicrobial effects have been documented in some herbal psychoactive substances and cannabis-related compounds (Appendino et al., 2008). However, the relatively lower antimicrobial activity against *Escherichia coli* may be due to the protective outer membrane of Gram-negative organisms, which limits penetration of antimicrobial agents.

The open field test demonstrated no statistically significant differences in locomotor activity, exploratory behaviour, line crossing frequency, movement duration, or rearing among the treatment groups. These findings suggested that VIP AK-47 Akuskura exerted limited effects on general locomotor activity at the tested doses. The absence of significant alterations in open field parameters may indicate that the substance did not markedly affect spontaneous exploratory behaviour or anxiety-related behaviour within the duration of exposure. Similar observations have been reported in studies involving cannabis exposure in experimental animals (Cawkwell et al., 2021).

However, despite the limited effects observed in the open field test, significant impairments were observed in the beam walk test and footprint analysis. The beam walk assessment revealed increased beam crossing time and increased frequency of hind foot slips in medium- and high-dose groups. These findings suggested impaired motor coordination and balance following exposure to VIP AK-47 Akuskura. The beam walk test is highly sensitive for detecting cerebellar dysfunction and motor deficits associated with neurotoxicity (Luong et al., 2011). Increased hind limb slips observed in this study may therefore indicate disruption of neuronal pathways involved in balance and fine motor coordination.

Similarly, footprint analysis demonstrated significant increases in forelimb and hind limb stride lengths among treated groups. Alterations in stride length may reflect compensatory gait changes associated with impaired neuromuscular coordination. Although increased stride length can occasionally indicate improved locomotor performance, in the present study the increase likely reflected abnormal gait adaptation secondary to motor impairment. These findings collectively suggested that VIP AK-47 Akuskura adversely affected gait regulation and motor coordination in a dose-dependent manner.

Histopathological examination of the cerebral cortex revealed vacuolation, pyknosis, neuronal degeneration, necrosis, and chromatolysis of pyramidal neurons in treatment groups. These findings indicated neuronal injury and neurotoxicity within cortical regions. Pyramidal neurons are the principal excitatory neurons of the cerebral cortex and play essential roles in motor integration, cognition, and information processing (Spruston, 2008). Degeneration of these neurons may therefore contribute to impaired motor coordination and altered neurological function.

The basal ganglia also demonstrated significant histopathological alterations

including enlarged pyramidal cells, nucleolar enlargement, dispersal of Nissl substances, vacuolation, and intense eosinophilia. Chromatolysis observed in the basal ganglia suggested neuronal stress and impaired protein synthesis secondary to axonal injury or toxic insult. The observed eosinophilia may indicate inflammatory responses and tissue injury associated with neurotoxic exposure. Similar neurodegenerative alterations have previously been associated with chronic exposure to psychoactive substances including cannabinoids, cocaine, and synthetic opioids (Cadet & Krasnova, 2009).

The cerebellum demonstrated Purkinje cell degeneration, pyknosis, karyolysis, neuronal depletion, and vacuolation in treatment groups. Purkinje cells are critical for coordination of balance, posture, motor learning, and fine motor control. Degeneration of these neurons may explain the impaired motor coordination observed during beam walk and footprint analyses. Pyknosis and karyolysis observed in cerebellar neurons are characteristic features of irreversible neuronal injury and cell death (Ibegbu et al., 2014).

The observed histological changes collectively suggested that VIP AK-47 Akuskura may exert neurotoxic effects on motor neuron-related brain regions. Cellular vacuolation observed in the study may represent reversible neuronal injury in some cells, whereas pyknosis, chromatolysis, and neuronal depletion indicated more severe and potentially irreversible damage. The mechanisms underlying these alterations may involve oxidative stress, excitotoxicity, neurotransmitter imbalance, mitochondrial dysfunction, or inflammatory responses induced by unidentified psychoactive constituents of the substance (Volkow et al., 2014).

Although some similarities were observed between VIP AK-47 Akuskura and other NPSs, the absence of chemical characterization remains a major limitation in interpreting these findings. The exact constituents responsible for the observed antimicrobial and neurotoxic effects remain unknown. Therefore, further analytical studies involving gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and toxicological profiling are necessary to identify the active compounds present in VIP AK-47 Akuskura and elucidate their mechanisms of action.

Overall, the findings of this study demonstrated that VIP AK-47 Akuskura altered oral microbial ecology, exhibited moderate antimicrobial activity, impaired motor coordination, and induced dose-dependent histopathological alterations in the cerebral cortex, basal ganglia, and cerebellum of mice. These findings suggest potential public health risks associated with the increasing abuse of VIP AK-47 Akuskura and highlight the urgent need for toxicological characterization, public health awareness, and regulatory surveillance of emerging psychoactive substances in Nigeria.

**Conclusion**

The present study demonstrated that VIP AK-47 Akuskura produced significant dose-dependent effects on oral microbial flora, motor coordination, and histopathological architecture of motor neuron-related brain regions in mice. Although the exact chemical composition and mechanism of action of the substance remain unknown, the findings suggest that prolonged exposure to VIP AK-47

Akuskura may pose serious neurological and oral health risks.

VIP AK-47 Akuskura altered oral microbial homeostasis by reducing beneficial oral commensals while promoting the proliferation of opportunistic microorganisms. The substance also exhibited moderate antimicrobial activity against selected oral pathogens, particularly Gram-positive bacteria. These findings indicate that the substance may contain biologically active phytochemical or psychoactive constituents capable of influencing oral microbial ecology.
Behavioral assessments revealed that VIP AK-47 Akuskura impaired motor coordination and gait patterns, particularly at medium and high doses. Significant increases in beam crossing time and hind foot slips suggested dysfunction of neural pathways involved in balance and locomotor regulation.

Histopathological analyses further demonstrated neuronal degeneration, cellular vacuolation, pyknosis, chromatolysis, Purkinje cell degeneration, nucleolar enlargement, eosinophilia, and necrosis within the cerebral cortex, basal ganglia, and cerebellum. These alterations indicate neurotoxicity and neuronal injury affecting critical brain regions responsible for motor coordination and higher neurological functions.

Although some observed effects share similarities with other new psychoactive substances and cannabinoid-related compounds, interpretation remains limited due to the absence of chemical characterization of VIP AK-47 Akuskura. Therefore, further studies involving toxicological profiling, phytochemical screening, gas chromatography-mass spectrometry (GC-MS), and molecular analyses are necessary to identify the active constituents and elucidate their mechanisms of action.
Overall, the findings of this study provide experimental evidence that VIP AK-47 Akuskura may constitute a significant emerging public health concern. There is an urgent need for regulatory monitoring, public awareness campaigns, and further scientific investigation into the health implications of this increasingly abused psychoactive substance in Nigeria.

**Recommendations**

1. Further studies should identify the chemical constituents of VIP AK-47 Akuskura.
2. Long-term toxicity studies should be conducted.
3. Public health awareness campaigns should educate youths about the dangers of Akuskura abuse.
4. Regulatory authorities should monitor and regulate emerging psychoactive substances.

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