Effects of Probiotics from Nigerian Fermented Foods on Reserpine Induced Depression in Adult Male Wistar Rat

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ABSTRACT

Background: *Depression is one of the most common mental health conditions, which affects the physical health of an individual. Reserpine slows the activity of the nervous system by slowing down the heart rate and blood vessels. Cassava and maize contain natural probiotics which have a protective and ameliorative effect on depression.*

Objective: *This study aimed at evaluating the effect of probiotics from local Nigerian fermented foods on reserpine-induced depression using experimental adult-male Wistar rats.*

Methods: *The yellow maize and cassava were washed thoroughly and fermented with submerged fermentation technique for three days and six days respectively. Probiotics were isolated using sub-culturing method. Twenty-five experimental rats were divided into five groups, Group 1- positive-control, group 2-negative-control, group 3, group 4, and group 5 were induced with reserpine. Group 2 no treatment while groups 3, 4 and 5 were treated with different doses probiotics from cassava and maize. Depression was diagnosed using the neurobehavioral tests index.*

Results: *Microbial analysis of fermented liquids showed the present of four strains of lactobacillus bacteria. Lactobacillus rhamnosus were most abundant strain in both liquids. Lactobacillus fermentum (7.51×103±0.14) and Lactobacillus acidophilus (6.78×103±0.16) were higher in cassava liquid and Lactobacillus plantarum (2.98×103±0.10) was higher in maize liquid. And reserpine administration caused significant decrease in the weight, dopamine, serotonin, glutathione and catalase activity level and treatment with probiotics from cassava and maize significantly improved these parameters.*

Conclusion: *This study provides evidence on the potential of probiotics derived from local Nigerian fermented foods in ameliorating reserpine-induced depression.*

Keywords: Depression, reserpine, probiotics, cassava, maize, fermentation.

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INTRODUCTION

Depression is a mood or emotional condition that is characterized by feelings of poor self-worth or guilt, sadness, emptiness, lack of interest in activities and a diminished capacity to enjoy life (1). Depression is associated with reduced monoamine neurotransmitter such as dopamine and serotonin (2).

Reserpine was an effective medication for treating hypertension and commonly used as the primary treatment as one study has shown (3). It was also effective in cases where hypertension was difficult to manage (3). However, it is now considered a secondary treatment option due to concerns about depression that emerged after its administration, leading to a significant decrease in its usage (4, 5). Probiotics have been studied for their therapeutic potential in a wide range of illnesses, including type 2 diabetes, obesity, irritable bowel syndrome,

asthma, malignancies, arthritis, and mental health disorders (6). Probiotics are a viable therapeutic intervention for mental diseases such as major depressive disorder due to their clinical success, lack of negative side effects, and lack of stigma. Within the past ten years, there has been a signi·cant increase in the number of clinical trials and research studies employing probiotics in the ·eld of mental health (7). Dietary intake of fermented foods and beverages from both plants and animals is signi·cant. These foods typically contain lactic acid bacteria (LAB) developed during fermentation; LAB naturally contain substances such organic acids, ethanol, or antibacterial substances with the ability to suppress spoilage organisms and pathogenic bacteria in fermented foods. Additionally, these bacteria—commonly known as probiotics—can adapt well to the spontaneous fermentation process and contribute to the health of both people and animals (8).

MATERIALS AND METHODS

Cassava tubers and maize grains were obtained from Ilishan-Remo Market in Ogun State

Fermentation of cassava and maize

Cassava and maize were fermented by submerged fermentation technique (9). The roots of cassava were peeled, washed, and cut into smaller bits. It was then submerged in water with a ratio of 1:3 for six days for fermentation. The fermented cassava were brought out from the water in which they were fermented and squeezed with cheese cloth to separate the pomace (residue) from the ltrate (liquid). The yellow maize was washed thoroughly to remove dirt. The maize was left in water for three days and was blended with an industrial food processor. It was sievedwith cheese cloth to separate the pomace (residue) from the ltrate (liquid). Therafter, sample of the two ·ltrates were taken for microbial analysis.

Microbial analysis and isolation of microorganisms for cassava and maize

The probiotics was isolated from cassava and maize fermentation. Cassava (5kg) was weighed and mixed with 250 ml of sterilized normal saline followed by serial dilution. Aliquot (1 ml) from the resulting mixture was placed in a duplicate sterile petri dish using the pour plate method. Nutrient agar (Product of Hi MEDIA) was used for the determination of bacteria. It incubated at 37°C for 24–48hours while potato dextrose agar (Product of Hi MEDIA) were used for the determination of fungus in the samples, incubated at 27°C for 48–72 h. The total number of available colonies obtained after incubation were counted and then expressed in CFU/g. The pure cultures of the isolates were acquired by sub-culturing on newly prepared agar plates.

Four Kilograms (4kg) of fermented corn maize were collected aseptically from the different buckets at 24 hours interval and homogenized in a mortar, it was cleaned with ethanol and passed over bunsen flame. The homogenized samples will be suspended in sterile 9 ml distilled water tubes and serially diluted (10 fold dilution). Dilutions (0.1 ml) of 10−3–10−5 were inoculated on sterile disposable petri dishes by pour plate method. The plates were labelled appropriately based on the media used; the media used include nutrient agar at 37°C for 24 hours and potato dextrose agar. Counts of bacteria was made on the media. Microorganisms isolated at 24 hours interval during the fermentation (steeping and souring) process was randomly picked based on colonial morphology differences and subcultured onto freshly prepared plates by streaking. The isolates were then puri·ed by repeated subculturing and stored in agar slants at 4°C.

Identification of microbes in cassava and maize

The identi·cation of isolates was carried out in four phases; cultural characterization which involves observing the microbial isolate growth characteristics and optimal condition for isolates this includes colony morphology, oxygen requirement and temperature requirements. The second phase is morphological characterization which involves the study of the structure and arrangement of the isolates, biochemical characterization is the third phase which involves the identifying the speci·c and metabolic activities of the isolates using catalase and oxidase tests, and lastly is the use of Analytical Pro·le Index (API) kits. These should be described fully to oxidase tests. Lastly, the use of analytical profile index (API) kits, which is a miniaturized standardized identification technique to determine the sub-specie of the microorganism.

Animal experiment

A total of twenty-·ve experimental rats were obtained from Babcock University animal facility and ethical approval was obtained from Babcock University Health Research and Ethical Committee (BUHREC) to carry out the research.

Data analysis

The data obtained were analyzed by a one-way analysis of variance (ANOVA) using GraphPad

Prism software (GraphPad Software, CA, USA). The normality of the data was checked prior to the ANOVA test in GraphPad Prism using D'Agostino-Pearson omnibus K2 normality test at a signi·cance level of 0.05. If the P value is greater than 0.05, the data is then said to be normal. If it is below 0.05, the data signi · cantly deviated from a normal distribution. The results were expressed as mean ± SD.

Experimental design

The twenty- ve-adult male Wistar rats were grouped into ·ve cages, each containing ·ve (5). The groupings were done in order of varying rat weights, adult male Wistar rats with a weight difference of ±7.1g grouped together.

This was done to administer adequate and correct dosage of the drugs in attempts to derive effective results for causative damages (reserpine) and ameliorative effects (probiotics). Administration of different doses of reserpine and liquid probiotics (shown in table 1) were carried out using a cannula for two weeks. Proper handling of the animals was practiced to ensure that harm was not caused to both the animal and the administrator. The experiment took a total time duration of 35 days (5 weeks). Seven days (one week) for animal acclimatization and 14 days induction of depression using reserpine only and 14 days for administration of the probiotics, a day was also used for neurobehavioral testing.

Method of sacrifice and biochemical analysis

Cervical dislocation was carried out To after which, the brain was dissected immediately and its homogenate stored for biochemical analysis to measure depression index, the levels of DA and 5-HT in the striatum were measured by ELISA according to the manufacturer's (USCN, Wuhan, China) instructions. A 30-mg portion of the homogenate was diluted in 300 μL normal saline (0.9%) for detection. We assayed DA and 5-HT in supernatant fluid using the ELISA kit (Zigma, USA) after centrifugation of homogenized tissue for 10 minutes at 20,000 g at 4°C. Dispensed antigen standards and samples were added to each well of 96-well plates precoated with primary antibodies. After adding biotin conjugate reagent and enzyme conjugate reagent into each well, the plates were incubated at 37°C for 60 minutes. Then the plates were rinsed 5 times with distilled water to wash buffer. Within 30 minutes of the chromogenic reaction, the absorbance was measured at 450 nm using a microtiter plate reader. The dead rats were properly disposed.

RESULTS

Table 2 shows the microbial analysis of the fermented cassava and maize liquids, all the four strains of Lactobacillus bacteria were present in both cassava and maize fermented liquids, with varying levels. Lactobacillus rhamnosus was the most abundant strain in both liquids, with levels of 5.07x10^3 ± 0.23 in cassava and 4.16x10^3 ± 0.60 in maize. Lactobacillus fermentum and Lactobacillus acidophilus were also present in both liquids, with higher levels in cassava compared to maize. And Lactobacillus plantarum was present in both liquids but at higher levels in maize compared to cassava.

Table 1: Experimental design

Group	Dosage
1	Distilled water
2	0.5ml reserpine
3	0.8ml reserpine + I ml probiotics from cassava and maize
4	0.6ml reserpine + 1ml of probiotics from maize
5	0.7ml reserpine + 1ml probiotics from cassava

Table 2: Microbial Analysis of Fermented Cassava and Maize Liquid

Sample Parameters	Cassava Liquid	Maize Liquid
Lactobacillus rhamnosu	$5.07\times10^3\pm0.23$	$4.16\times10^3\pm0.60$
Lactobacillus fermentum	$7.51\times10^3\pm0.14$	$2.50\times10^3\pm0.60$
Lactobacillus plantarum	$2.30\times10^3\pm0.57$	$2.98\times10^3\pm0.10$
Lactobacillus acidophilus	$6.78\times10^3\pm0.16$	$6.78\times10^3\pm0.08$

Figure 1 shows the relative body weights of the rats across experimental groups. The result shows a signi·cant decrease in relative body weight of group 2 compared to other groups. The relative body weights of the groups intoxicated with reserpine and then treated with probiotics from maize and cassava signi·cantly increased in comparison with the reserpine groups. (*=p<0.05, **=p<0.01, ***=p<0.001).

Bars showing the effect of probiotics on the Dopamine level of reserpine induced toxicity is shown in Figure 2. Dopamine level in the reserpine intoxicated group was signi·cantly lower when compared across experimental groups. The normal dopamine levels in rats ranges between 0.15-0.32 µmol/mg. Treatment with cassava and maize which contains probiotics increased the dopamine level that was decreased by reserpine intoxication. (*=p<0.05, **=p<0.01, ***=p<0.001).

Figure 3 displays the effect of probiotics on the serotonin level of reserpine induced toxicity in rats. Serotonin level in the reserpine intoxicated group was signi·cantly reduced when compared across experimental groups. Treatment with cassava and maize which contains probiotics increased the serotonin level that was lowered by reserpine intoxication.

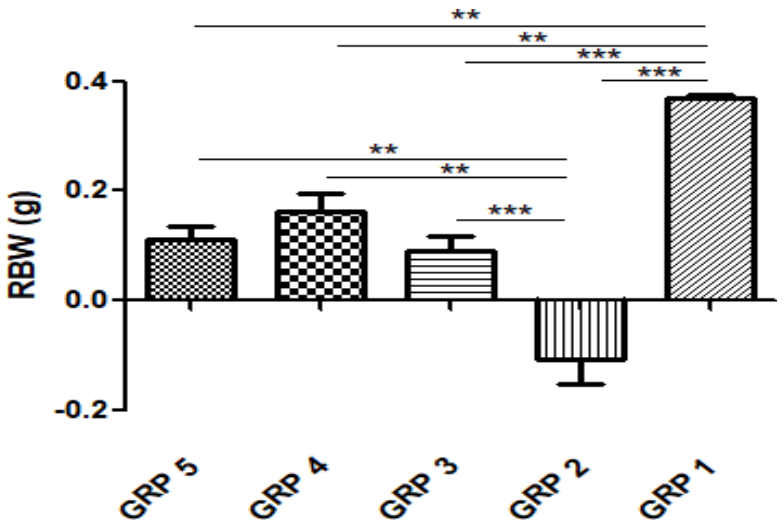

Figure 1: Relative Body Weight of Experiment Rat

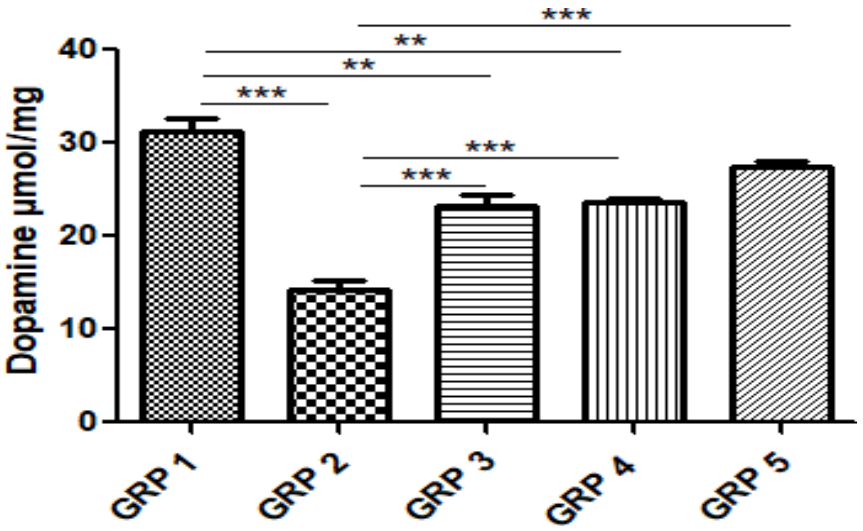

Figure 2: Dopamine level of experimental rats

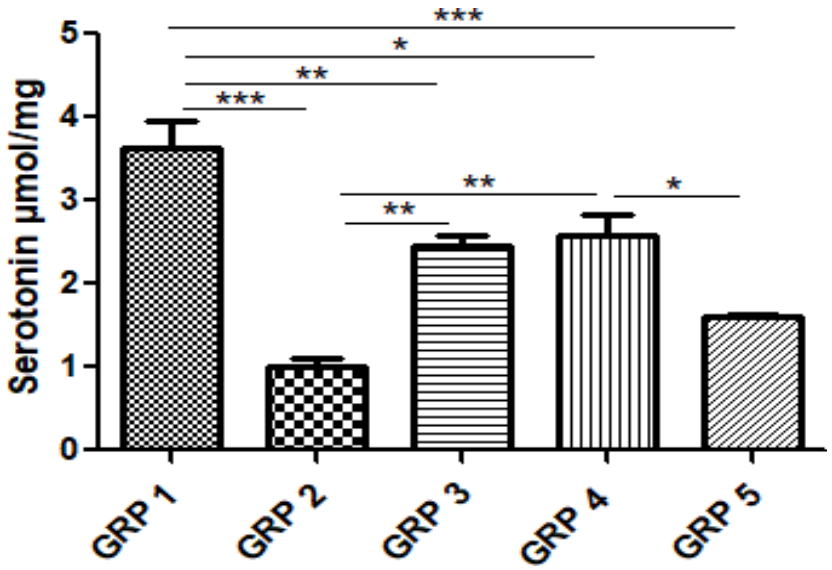

Figure 3: Serotonin level of experimental rats

Figure 4 displays the cytoarchitecture, cellular assortment, and Nissl granules integrity of the amygdala across experimental groups investigated with Crysl Fast Violet stain at low and high magni·cation. The pyramidal neurons population in control group (GRP 5) were normal with regular cell body and processes characterized by chromatogenic Nissl substances and regular neuronal density (yellow arrows). Reserpine intoxication (GRP 4) resulted to chromatolytic pyramidal neurons with degenerative features (pyknosis and necrosis) scattered within the neuropil in the amygdala. Cytoplasmic inclusions, nuclear materials, Nissl substances and neuronal membrane are greatly compromised (red arrows). Treatment of rats exposed to reserpine with probiotics from cassava and maize (GRP 1, 2, and 3) improvesigni· cantlytheamygdala cytoarchitecture as pyramidal neurons in these groups showed neuronal features that is similar to that of the control groups (yellow arrow).

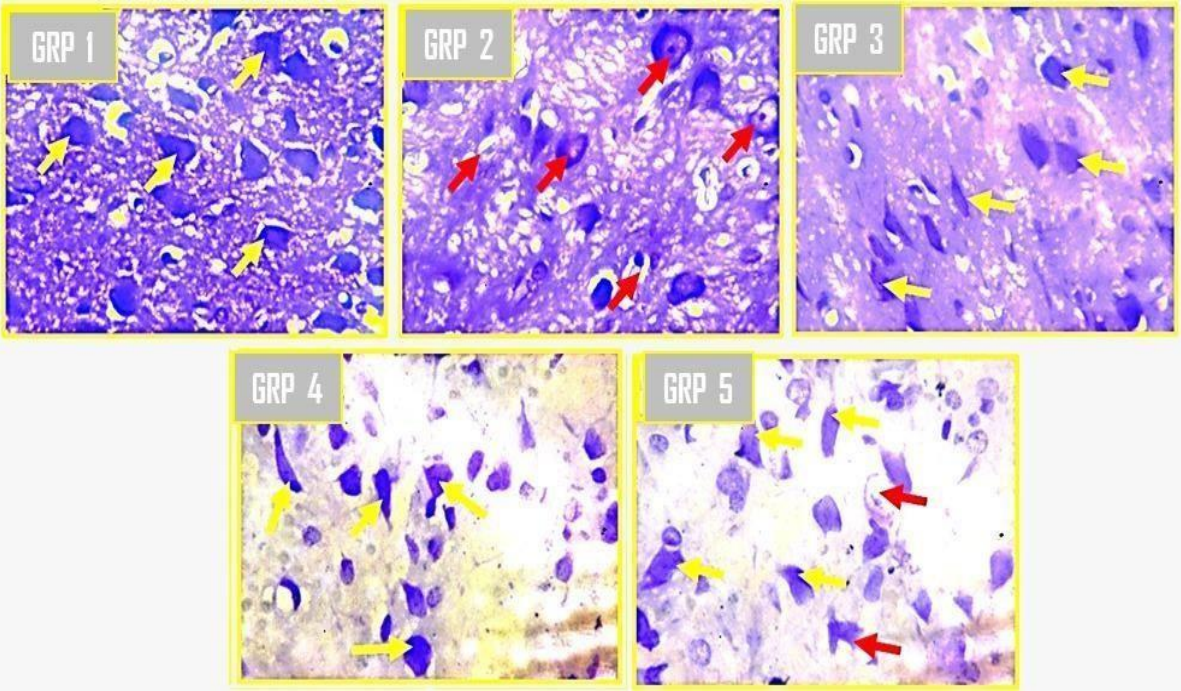

Figure 4: Histology of the experimental rats

DISCUSSION

To determine the protective role of probiotics from Nigerian fermented foods on reserpine-induced depression, probiotics content of fermented cassava and maize liquids were analyzed, weight, serotonin, dopamine and histological features were investigated using experimental rats.

All the four strains of Lactobacillus bacteria were present in both cassava and maize fermented liquids (Table 2), with varying levels (11). L.rhamnosus was the most abundant strain in both liquids but higher in cassava than in maize (12, 13). Lactobacillus fermentum and Lactobacillus acidophilus were also present in both liquids, with higher levels in cassava compared to maize and Lactobacillus plantarum were present in both liquids but at higher levels in maize compared to cassava (14, 15, 16).

There is a signi·cant decrease in relative body weight of group 2 (Figure 1) compared to other groups and the groups intoxicated with reserpine and then treated with probiotics from maize and cassava had signi·cant increase in the relative body weight in comparison with the reserpine groups (17, 18).

The results of dopamine and serotonin assessment (Figure 2 and Figure 3) shows that there was a signi·cant decrease in dopamine and serotonin level in experimental groups exposed to reserpine, which indicate depression and other neuro disorder. The reduction of serotonin and dopamine level due to blockage of mono amines caused by reserpine intoxication is demonstrated in research work (19). Reactive oxygen species (ROS) can potentially contribute to the pathophysiology of depression through diverse mechanisms, including tissue damage, inflammation, neurodegeneration, autoimmune responses triggered by tissue damage, and apoptosis (20). The brain's heightened vulnerability to oxidative stress (OS) stems from its elevated oxygen consumption, greater lipid content, and less robust antioxidative defence mechanisms (21).

Histopathological studies (Figure 4) using Crystal Fast Violet stain revealed distinct differences in the cytoarchitecture, cellular assortment, and Nissl granules integrity of the amygdala across experimental groups. In the control group, the amygdala exhibited normal cytoarchitecture, with pyramidal neurons characterized by regular cell bodies and processes.

These neurons displayed chromatogenic Nissl substances, indicating intact protein synthesis machinery, and exhibited a regular neuronal

density. In contrast, reserpine intoxication resulted in signi cant histopathological alterations in the amygdala. Pyramidal neurons exhibited chromatolysis, characterized by the loss of Nissl substances and the presence of degenerative features such as pyknosis and necrosis. These chromatolytic neurons were scattered within the neuropil, indicative of neuronal damage and disruption of the normal cellular arrangement. Furthermore, the cytoplasmic inclusions, nuclear materials, Nissl substances, and neuronal membrane integrity were severely compromised, suggesting extensive cellular damage, this was also demostrated in a research work by (22). A research (23) shows histopathological alterations due to reserpine treatment and assessment using the behavioural paradigm.

Histological examination of brain tissue exposed to reserpine reveals notable alterations in neuronal structure and organization. One study (24) demonstrated that reserpine administration led to a signi cant reduction in the number of neurons in the hippocampus. Furthermore, the study also reported abnormal cellular morphology, such as shrinkage and irregularity in the shape of neurons. Nissl granules, which are basophilic bodies found in the cytoplasm of neurons, play a crucial role in protein synthesis and overall neuronal function. Reserpine-induced neurotoxicity can result in injury to these granules. Research conducted by (25) showed that reserpine exposure caused a decrease in the intensity and distribution of Nissl granules within neurons. This disruption in Nissl granules indicates impaired protein synthesis and cellular dysfunction. Remarkably, treatment of rats exposed to reserpine with probiotics derived from cassava and maize showed a signi cant improvement in the cytoarchitecture of the amygdala. Pyramidal neurons in these groups displayed neuronal features similar to those observed in the control group, including regular cell bodies, processes, and chromatogenic Nissl substances. This suggests that the administration of cassava and maize-derived probiotics protected against reserpine-induced neurotoxicity and preserved the structural integrity of the amygdala.

Previous studies have demonstrated the neuroprotective effects of probiotics in various brain regions. Probiotics have been shown to modulate neurotransmitter levels, reduce oxidative stress, and attenuate neuroinflammation, which could potentially contribute to their protective effects on neuronal architecture, although the exact mechanisms underlying the observed improvements in the amygdala cytoarchitecture in response to probiotics are yet to be elucidated, these ndings are consistent with previous research demonstrating the bene cial effects of probiotics on brain health (26, 27, 28, 29, 30)

CONCLUSION

Probiotics from cassava and maize have potential therapeutic bene ts against reserpine-induced depression. The use of these natural probiotics strain may have a collaborative effect, making them a promising option for the ameliorative and protective effect on depression.

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