



Effect of Ethanolic Extract of a Herbal Mixture, AjuMbaise, on the Histomorphology of the Hippocampus and the Hypothalamus of Adult Wistar Rats Exposed to High-Fat Diet

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ABSTRACT

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High fat diet (HFD) has been reported to cause oxidative stress and brain damage. Reports show that a herbal mixture, AjuMbaise, is rich in phytonutrients with vast medicinal values. The study investigated the effects of ethanolic extract of a herbal mixture, AjuMbaise, on the histomorphology of the limbic system of adult Wistar rats exposed to HFD. Fresh samples of AjuMbaise procured from Afor-Enyigugu Market, Amuzi, Aboh-Mbaise L.G.A., of Imo State, Nigeria were air-dried, blended and macerated in 95% ethanol for 72 hours. The extract was concentrated and stored in an air-tight container in a refrigerator. Twenty-five Wistar rats were divided into 5 groups, 1-5 (n=5) each. Group 1 received normal saline, group 2 received HFD and normal saline, while groups 4, 5 and 6 received HFD and 200mg/kg, 400mg/kg and 800mg/kg of the herbal mixture, respectively for 49 days. All groups received feed and water *ad libitum*. On day 50, the rats were anaesthetized with 50 mg/kg thiopental sodium and aortic perfusion fixation with 4% paraformaldehyde was carried out. The brains were dissected out and the hippocampus and hypothalamus were harvested, fixed in Bouin's fluid and processed for Nissl stain. Group 2 showed hippocampal atrophy and neuronal degenerations in the hypothalamus while groups 3, 4 and 5 showed progressive recovery from the hippocampal and hypothalamic degenerations. The results show that ethanolic extract of the herbal mixture, AjuMbaise, can reverse the deleterious effect of HFD on the Hippocampus and the Hypothalamus in a dose dependent manner.

Keywords: AjuMbaise, High Fat Diet, Limbic System.

Introduction

Herbal plants have been used in medical practice since antiquity as the primary remedy in traditional medicine.¹ Traditional herbal practitioners have described the therapeutic efficacy of many indigenous plants for various diseases, in addition to their ancient historical uses.² Traditional healers claim that certain plants are more effective as well as safer than synthetic antibiotics in treating infectious diseases. However, most users of these medicinal plants do so for its affordability and availability. Such plants have parts such as leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds that are used in the control or treatment of a disease condition.³ According to the World Health Organization, 80 percent of the population in developing countries, rely on herbal medicines to meet the majority of their health needs.⁴ It is estimated that more than 90% of pregnant women in Africa consume plants for uterotonic purposes and that at least 56 plant species are currently used for such purposes.⁵

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Various phytochemical constituents of foods and beverages, particularly a class of phytochemicals known as flavonoids, have received a lot of attention.^{6,7,8} Some dietary intervention studies in humans and animals, particularly those involving foods and beverages derived from grapes, cocoa, and so on, have shown that they improve vascular function and mental performance.⁹ In Nigeria, the use of plants and their natural products as extracts or infusions is a common practice in the treatment and management of diseases.¹⁰ "AjuMbaise" is a popular decoction from the Igbo people of South-East Nigeria. The polyherbal cocktail is native to the Mbaise Community in Mbaise Local Government Area of Imo State, Nigeria.² as a mixture of various plant leaves and stems tied together with twine. Ogueke *et al.*,¹¹ identified the following plants as components of AjuMbaise: *Jatropha curcas* (Barbados nut, purging nut), *Cnestis ferruginea*, *Combretum racemosum* (Bushwillow tree), *Chrysophyllum albidum* (African Star apple), *Heterotis rotundifolia* (rock rose, Spanish shawl), *Sphenocentrum jollyanum* (Akerejupon in Yoruba), guava, etc. The plants are used in a variety of ways, including poultices, concoctions of various plant mixtures in porridges and soups, through oral, nasal, topical (lotions, oils, or creams), bath, or rectal "enemas".³ It is traditionally thought to be a powerful remedy for weight loss, ovarian cysts, diabetes, irregular menstrual cycles, and eczema, etc. The use of AjuMbaise is increasing especially among the female folks because of the wide range of conditions for which it is said to be efficacious. Most women of all ages therefore use it as a means of keeping to shape as well as maintaining the wellbeing of their internal organs. Report shows that the cocktail herbal mixture of AjuMbaise contains a tremendous amount of phytochemicals that possess numerous therapeutic potentials.^{2,12} Qualitative phytochemical analyses of the ethanol extract of AjuMbaise herbal mixture shows the presence of

alkaloids (8.69%), flavonoids (19.10%), glycosides (6.86%), hydrogen cyanide (0.92%), phenols (31.56%), saponins (0.83%), steroids (0.94%), tannins (16.80%), and terpenoids (14.31%).¹²

Plant-derived polyphenolic compounds are promising nutraceuticals for the treatment of a variety of disorders, including cancers, diabetes, aging diseases, urinary tract infections, obesity, cardiovascular, neurological, periodontal, and neoplastic diseases.^{13,14} The high nutritive value of AjuMbaise polyherbal mixture due to its substantial amount of minerals and multivitamins has been reported,^{15,11} as well as its potency as cardioprotective and hypolipidemic,¹⁶ but at the same time report that prolonged use of doses of AjuMbaise above 400mg/kg body weight can be hepatotoxic and nephrotoxic.¹⁶ AjuMbaise polyherbal mixture has been in wide use as treatment as well as supplements for better health especially among women amidst the claim that it controls their weight and maintains their shape. There is therefore the need to explore its safety on the brain. This study evaluated the effects of ethanolic extract of a herbal mixture, AjuMbaise, on the histomorphology of the hippocampus and the hypothalamus of adult female Wistar rats exposed to high-fat diet

Materials and Methods

Experimental Animals

A total of 25 adult female Wistar rats were procured from the Animal Facility of the College of Medicine University of Nigeria, Enugu Campus (UNEC), and were acclimatized to the conditions of the animal house of the Department of Anatomy, UNEC for 14 days.

Preparation of the Lard

Methods: Fat tissue was cut from the fat deposits in the pig abdominal walls, cut into pieces, washed thoroughly in running water and put inside a cooking pot. Water was added and boiled under constant temperature for 30 minutes. It was allowed to cool at room temperature, and the oil layer was sieved using a regular sieve cloth, poured in a plastic container, and stored in a refrigerator overnight.

Result: The content after refrigeration, gave a white semi-solid organic substance known as Lard very rich in lipids (fats).

High Fat-diet preparation

High fat diet (HFD) was prepared by mixing the prepared lard with sucrose and normal animal feed (broiler finisher pellets feed). The composition of the high-diet was calculated in the ratio of 3:1:1 i.e. animal feed, Lard, and sucrose respectively, and was carefully harmonized according to Nnadiukwu et al.¹³

Collection of plant samples

Fresh samples of the plants that make up AjuMbaise were procured from the dealers at Afor-Enyigogu market in Amuzi, town in AbohMbaise L.G.A., of Imo State, Nigeria. The procurement of plant samples took place in June 2021.

Extraction of Herbal materials

The plant materials were washed, air-dried for 72hours, weighed, and blended to powder. Powdered sample weighing 330g was macerated in 3,000ml of 95% ethanol for 72hours after which it was sieved using a muslin cloth and afterward filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary evaporator at 45°C and afterward placed on a thermostatic water bath for further drying. The residue was collected, weighed, kept in a sterile bottle, and stored in a refrigerator until required for usage. The 300g of the plant materials yielded 12.5g residue.

Experimental Design

The 25 female Wistar rats were divided into 5 groups, with 5 rats in each group.

Group 1: received normal diet throughout the experiment.

Group 2: received high-fat diet for 21days, then normal saline for 28days

Group 3: received high-fat diet for 21days, then 200mg/kg of AjuMbaise for 28days.

Group 4: received high-fat diet for 21days, then 400mg/kg of AjuMbaise for 28days.

Group 5: received high-fat diet for 21days, then 800mg/kg of AjuMbaise for 28days.

Termination of Experiment

On day 50 of the experiment, the rats were anaesthetized with 50 mg/kg thiopental sodium and aortic perfusion fixation with 4% paraformaldehyde was carried according to the method of Finbarrs-Bello *et al.*¹⁷ The brains were dissected out and further fixed in 4% paraformaldehyde overnight and then later processed for Nissl stains. Sections were deparaffinized in 3 changes of xylene for 10 minutes each. Sections were stained in 0.1% cresyl violet solution for 5-10 minutes. Sections were quickly rinsed in distilled water. Each section was then differentiated in 95% ethyl alcohol and was viewed under the light microscope after every minute for best result. Sections were then dehydrated in 2 changes of absolute alcohol for 5 minutes and covered with glass coverslips using DPX Mountant. The photomicrographs were captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, USA.). The animals were handled with care Ethical approval was obtained from the the College of Medicine Research Ethics Committee (COMREC) University of Nigeria, Ituku/Ozalla, Enugu with protocol number UN/CM/REC/LABAN/054/01/2021 issued on 15th April, 2021.

Results and Discussion

The results are shown in the photomicrographs of the hippocampus (Figure 1-5 and hypothalamus (Figure 6-10).

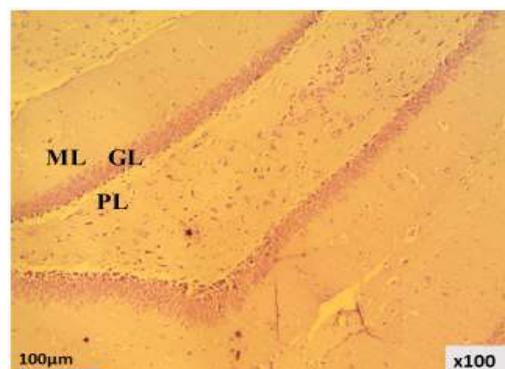


Figure 1: Photomicrograph of group 1 of the Hippocampus showing the cells of the CornuAmmonis (CA1-CA4) and the pyramidal cell layers (ML, GL, PL) with normal histomorphology, supporting cells were also seen intact with the pyramidal cells. Nissl stain

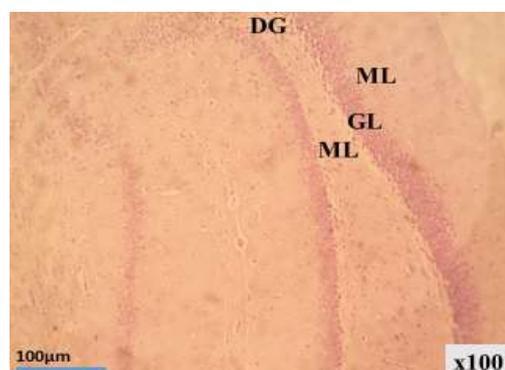


Figure 2: Photomicrograph of group 2 of the Hippocampus showing the pyramidal cell layers with a hippocampal atrophy (DG and the *Cornu Ammonis*). Nissl stain.

Group 1(control) showed normal histomorphology of the hippocampus (Figure 1) and hypothalamus (Figure 6). Group 2 (HFD only) showed atrophy at the dentate gyrus and Cornu Ammonis (CA1-CA4) cells of the hippocampus (Figure 2) and alterations in the paraventricular nuclei of the hypothalamus (Figure 7). Group3 (HFD + Low dose herbal extract (200mg/kg)) showed mild hippocampal atrophy and reduced density of neuronal cells of CornusAmmonis, and mild alteration in the morphology of the paraventricular nuclei of the hypothalamus. Group 4 (HFD + medium dose herbal extract (400mg/kg)) showed increased dendrites of the the neuronal cell at the dentate gyrus and cornusAmmonis of the hippocampus (Figure 4), and slight alteration in the morphology of the paraventricular nuclei of the hypothalamus (figure 9). Group 5 (HFD + high dose herbal extract (800mg/kg)) showed remarkably increased density of the neurons at the CornusAmmonis and dentate gyrus of the hippocampus, and intact nuclei and supporting cells of the hypothalamus. High body fat has been implicated in a number of poor health conditions including obesity, Alzheimer's disease and alteration of brain structure and neurotransmitters.¹⁸Businaro *et al*,¹⁹ and Mohamed *et al*,²⁰ suggested that consumption of high fat diet can alter brain structure and function as well as neurotransmitters. In this study, group 3 (fed with high fat diet) showed altered histological changes in the hippocampus with degenerating neuronal cells; the hypothalamus of the high fat diet exposed rats also shows scanty cytoplasm. These alterations in the histoarchitecture of the hippocampus and hypothalamus of group 3 are clear when compared with group 1 (control). These alterations could be attributed to the effect that high fat diet can affect the brain function and structure,¹⁸⁻²⁰ and agrees with the findings of Zachary and Tamashiro,²¹ and Moraes *et al*,²² which suggests oxidative stress as the underlying mechanism for brain damage caused by high fat diet. Groups 3, 4, and 5 (treated with 200mg/kg, 400mg/kg, and 800mg/kg respectively) showed evidence of improvement in the cytoarchitecture of the hippocampus (Figure 3, 4 and 5 respectively) and hypothalamus (Figure 8, 9 and 10) respectively. The observed improvement was better at the higher dose. Group 5 showed the best outcome in both the hippocampus and hypothalamus (Figure 5 and 10) indicating that the efficacy of the extract of AjuMbaise polyherbal against high fat diet induced neuronal damage on the hippocampus and hypothalamus. Comparison across the treated groups shows that group 5 which received a high-fat diet and high dose (800mg/kg of extract), the degenerated neurons from the hippocampus and the hypothalamus shows a marked progressive improvement. Therefore it can be inferred that the degeneration caused by high-fat diet can be ameliorated by the treatment with ethanolic extracts. The comparative appraisal shows that the ameliorative effect of ethanol extract of the herbal mixture as observed in groups 3-5 clearly could be said to be dose dependent because of the marked progressive improvement of the histoarchitecture as the dose increases. Previous studies have demonstrated that the component plants that make up the polyherbal mixture are rich in polyphenolic compounds which are very potent antioxidants,¹²⁻¹⁴. It is therefore logical to attribute the ameliorative effects observed in the treated groups (groups 3, 4 and 5) to the antioxidant properties of the extract of the herbal mixture.

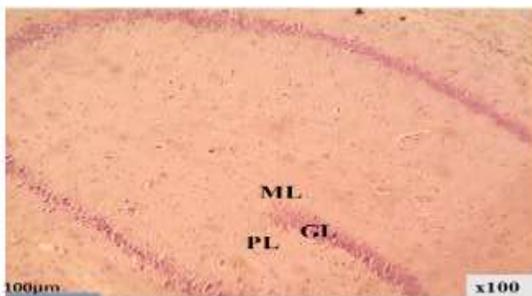


Figure 3: Photomicrograph of group 3 of the Hippocampus showing the pyramidal cell layers, with a mild Hippocampal atrophy. Density of the neuronal cells of the Cornu Ammonis were seen reduced. Nissl stain.

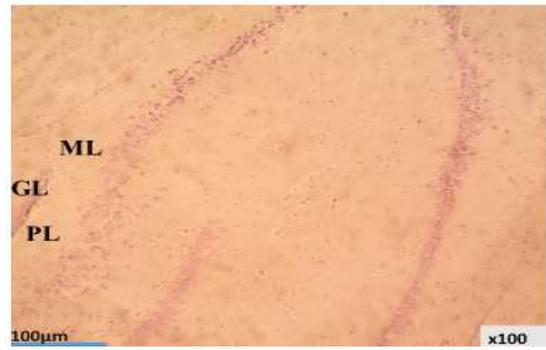


Figure 4: Photomicrograph of group 4 of the Hippocampus the dentate gyrus and the CornuAmmonis neuronal cell layers, shows an increasing density. Nissl stain.



Figure 5: Photomicrograph of group 5 of the Hippocampus, the density of the neuronal cell at the *Cornu Ammonis* were greatly increased. Nissl stain.

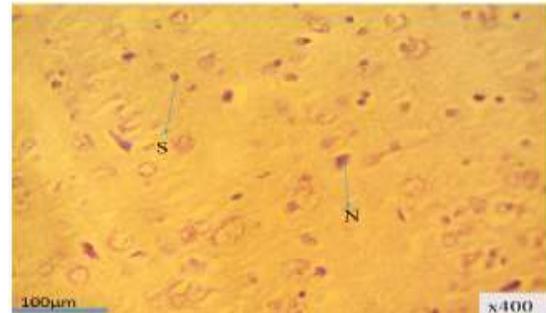


Figure 6: Photomicrograph of group 1 of the Hypothalamus showing no clear alteration in morphology of the hypothalamic nuclei, and the neurons (N) and the supporting cells were also seen intact. Nissl stain.



Figure 7: Photomicrograph of group 2 of the Hypothalamus showing an alteration in morphology of the hypothalamic nuclei, (paraventricular nucleus), with an infarction of the neurons (N). Nissl stain.

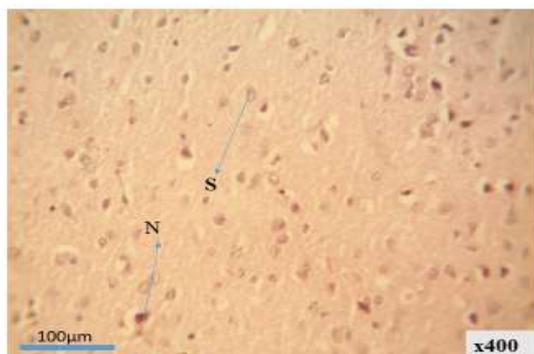


Figure 8: Photomicrograph of group 3 of the Hypothalamus showing an alteration in morphology of the hypothalamic nuclei, (paraventricular nucleus), with an infarction of the neurons (N). Nissl stain.

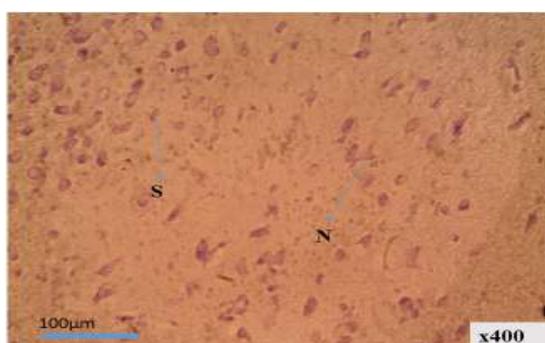


Figure 9: Photomicrograph of group 4 of the Hypothalamus showing an alteration in morphology of the hypothalamic nuclei, (paraventricular nucleus), with a mild infarction of the neurons (N). Nissl stain.

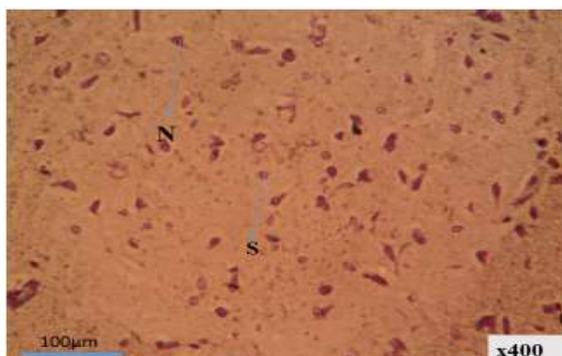


Figure 10: Photomicrograph of group 5 of the Hypothalamus showing no clear alteration in morphology of the hypothalamic nuclei, and the neurons (N) and the supporting cells were also seen intact. Nissl stain.

Conclusion

From the results in this study, we have demonstrated that AjuMbaise herbal cocktail can be effective in ameliorating and palliating neuronal cell damage caused by a high-fat diet on the hippocampus and the hypothalamus of adult Wistar rats.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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