NUTRITIONAL ASSESSMENT AND SUB-ACUTE TOXICITY STUDY IN WISTAR RATS FED WITH A COMPOUNDED DIET OF ROASTED PLANTAIN AND FISH SOLD IN OTUOKE

Abstract

The widespread consumption of roasted plantain and fish (*Bolle*), a common delicacy in Bayelsa State has raised concerns over potential health risks associated with food processing contaminants such as acrylamides, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). The roasted plantain and fish are usually prepared by placing the peeled plantain and the dressed fish on a wired gauze and placed over a burning charcoal. The subacute toxicity study of this diet was investigated using twenty adult male rats averagely weighing 117.22 ± 4.48 g. The rats were divided into four groups of five rats per group. Group A (Control), was fed with the standard rat chow, Group B; was fed with only the roasted plantain, Group C; was fed with only the roasted fish, and Group D; was fedwitha combination of the roasted plantain and fish. The rats were given free access to the feed and portable water *ad-libitum* throughout the experimental protocol. Body/organ weight data were recorded before and after the completion of the experimental protocol. On day 15^{th} of the experimental protocol, the rats were euthanized and blood specimens were obtained by cardiac puncture for biochemical analysis. The liver, kidney and heart were dissevered instantaneously and weighed. Findings from the study showed that the roasted plantain-fed group had significantly reduced body weight gain, and increased liver and kidney to body weight ratio ($p > 0.05$). The plasma enzyme activity (AST, ALT and ALP) of the plantain-fed group were also significantly increased ($p < 0.05$) while other groups showed non-significant differences ($p > 0.05$) with the control group. The antioxidant status (CAT, SOD and GPx), haematological parameters (RBC, PCV, Hb, MCV, MCH, MCHC and platelet) of the plantain-fed groups were also significantly decreased ($p < 0.05$), while other groups had non-significant changes ($p > 0.05$) when compared to the control group. Malondialdehyde levels in the roasted plantain-fed group were also significantly increased ($p < 0.05$).

Keywords: Antioxidants, food contaminants, plasma enzymes, street food, toxicity,

Introduction

Food is a fundamental aspect of human existence and is pivotal in maintaining overall health and well-being. Food is considerably more than a supplier of nourishment, it brings people together, shapes culture, and sways economics and environmental renewability. Recently there has been an upsurge in the patronage of street foods. Street foods are ready-to-eat foods and beverages prepared and vended in public places like markets, streets, or food trucks [1]. The rising popularity of street foods among the populace can be attributed to their low price, handiness, cultural appeal and evolving societal trends. Street foods contribute meaningfully to the local economy, provide jobs and incomes to vendors and boost local tourism.

One of Nigeria's most commonly consumed street foods, especially in Bayelsa State, is the roasted plantain, fish and sauce recipe popularly referred to as '*Bolle'*.The roasted plantain is made by placing the peeled plantain on a wired gauze and placed over a burning charcoal, the fish after proper dressing which involves the removal of guts and other inedible parts, it is thoroughly washed, salted and marinated in a combination of local spices is placed on the wired gauze and smoked over the same burning charcoal used for the plantain. The char-grilled plantain and fish are often kept in a spot of the roasting gauze with very low heat intensity, on the arrival of a potential buyer it is immediately re-heated and sauced with stew prepared from palm oil, pepper onion, vegetables and other local culinary spices for added taste and flavour.

Plate 1A: Roasted plantain Plate 1B: Roasted plantain &fish Plate 1C: Roasted fish

Plate 1A-C: Taken from the point of purchase

The method of preparing this recipe has been reported to cause food contamination [2, 3]. Food contamination is a significant public health concern, with various contaminants affecting the quality and safety of food products [4]. The method of preparation of the roasted plantain and fish involves direct exposure of the fresh plantain and fish to charcoal heat on a grid or wire mesh and this processing technique has been associated with severe contamination with several classes of persistent organic pollutants (POP) such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), brominated flame-retardants (BFRs), organochlorine compounds (OCs) perfluoro alkyl substances (PFAS) and chlorinated flame retardants (CFRs) [5].

The widespread consumption of roasted plantain and fish, popular delicacies in Bayelsa State raises concerns over dietary exposure to these POPs which may result in adverse toxicological effects like carcinogenicity, immune system suppression, reproductive and developmental toxicity, endocrine disruption, liver damage etc. on humans [6].Despite the known toxicological effects of these compounds, there is limited research on the specific

risks they pose to consumers, hence this study is aimed at investigating the proximate composition of this recipe and its subacute effect on some biochemical biomarkers of toxicity index.

Materials and Methods

Plantain and Fish Source

The roasted plantain and fish samples were purchased at the West Campus gate of the Federal University Otuoke, Ogbia Local Government Area of Bayelsa State, Nigeria.

Determination of Proximate Composition

The percentages of moisture, protein, fat, carbohydrate, ash and fibre contents of the roasted samples were assayed according to the standard methods of the Association of Official Analytical Chemists [7]. Moisture was determined by drying the samples at 130℃ in a hot air oven (DHG 9140A) until a constant weight was attained. The crude protein content was estimated by the micro-kjeldahl method and a nitrogen conversion factor of 6.25 was used. The fat content was estimated by the Soxhlet extraction method with ethyl ether. The ash content of the samples was estimated gravimetrically after the incineration of the samples at 550℃ for 2 hrs in a muffle Furnace (Model SXL). Fibre was estimated by enzymatic gravimetric technique, and the carbohydrate content was estimated as the difference of $[100 - (\%\,moisture + \%\,protein + \%\,fat + \%\,ash + \%\,fibre)]$. The caloric values of the samples were estimated using the method of Kpomah *et al.*[8].

Experimental Animals

Twenty healthy, male albino rats averagely weighing 117.22 ± 4.48 g were used. The animals were kept in a room which was clean and well-ventilated at a temperature between 28-30℃, under a natural dark/light cycle with free access to standard rat chow and water *ad-libitum* during the period of acclimatization which lasted for one week.

Animal Grouping

The twenty adult male rats were divided into four groups with five rats per group. Group A (Control); were fed with the standard rat chow, Group B; was fedwith only the roasted plantain, Group C; was fed with only the roasted fish, and Group D; was fed the combination

of the roasted plantain and fish in a ratio of 70:30 by weight. The rats were given free access to the fed and portable water *ad-libitum* throughout the experimental protocol.

Weekly Cage Side Surveillance for Physical Signs of Toxicity

This was done by physical examination of the rats from the cage sides for overt signs of toxicity on days 1, 7 and 14 respectively.

Animal Handling Procedure

On day 15th of the experimental protocol, the rats were euthanised using a diethyl ether chamber, blood specimens were obtained by cardiac puncture into two different sets of sample bottles. The first, into plain sample bottles and the blood specimen in it, was allowed to stand for 20 minutes for coagulation to occur, afterwards, the blood specimen was centrifuged at 2000 rpm for 10 minutes and the supernatant (serum) was collected and stored in the refrigerator before biochemical assay. The second set was collected into EDTA bottles and used for full blood count. The liver, kidney and heart were dissevered instantaneously and weighed.

Body weight before and after the feeding protocol

Rats in the respective groups were weighed before the commencement of the feeding protocol and on the 15th day after the termination of the feeding protocol. The percentage change in body weight was evaluated by the method of Kpomah and Arhoghro[9] andKpomah*et al.*[10]as expressed in the Equation 1

$$\%\ change\ in\ body\ weight\ = \frac{final\ body\ weight - initial\ body\ weight}{initial\ body\ weight} \times 100\% \ldots\ldots (1)$$

Organ (kidney, liver and heart) as the ratio of body weightbefore and after feeding protocol

The resection of the organs (liver, kidney and heart) was performed and weighed immediately. The organ ratio was evaluated by the method of Kpomah and Arhoghro[9] and Kpomah*et al.*[10]as expressed inEquation2

$$Organ\ ratio\ (\%)\ =\ \frac{weight\ of\ organ\ (g)}{body\ weight\ (g)} \times 100\% \ldots\ldots (2)$$

Assay Kits and Reagents

Assay kits for biomarkers of plasma enzyme activity and antioxidants are products of Randox Laboratories Ltd., United Kingdom. All other reagents/chemicals were purchased from reputable suppliers and are of analytical grade.

Plasma enzyme activity

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the methods of Reitman and Frankel [11] with slight modifications as reported by Kpomah *et al.*[12].Alkaline phosphatase (ALP) was determined by the method of REC [13] with slight medication Kpomah *et al.*[12].

Antioxidant status

Catalase CAT) activity was determined by the method of Kaplan and Grove [14], superoxide dismutase (SOD) by the method of Misra and Fridovich[15], glutathione peroxidase (GPx) by the method of Chance and Maehly[16] and lipid peroxidation as depicted by malondialdehyde levels was determined by the method of Buege and Aust [17].

HaematologicalAnalysis

Full blood count was done using an automated haematology analyzer, Sysmex XN-1000 (Sysmex Corporation), Kobe-Japan

Statistical Analysis

Experimental values were expressed as means ± standard deviation (n = 5). To determine differences between the groups studied, a one-way analysis of variance (ANOVA) was used to compare the group means, $p < 0.05$ was considered statistically significant. SPSS for Windows version 23.0 (IBM Corp, USA) was used for the statistical study and the charts were plotted using GraphPad Prism 8.

Results and Discussion

Proximate and Caloric Value of Roasted Plantain andFish

The proximate composition of roasted plantain and fish quantitatively indicated the presence of the major food components as presented in Figure 1.

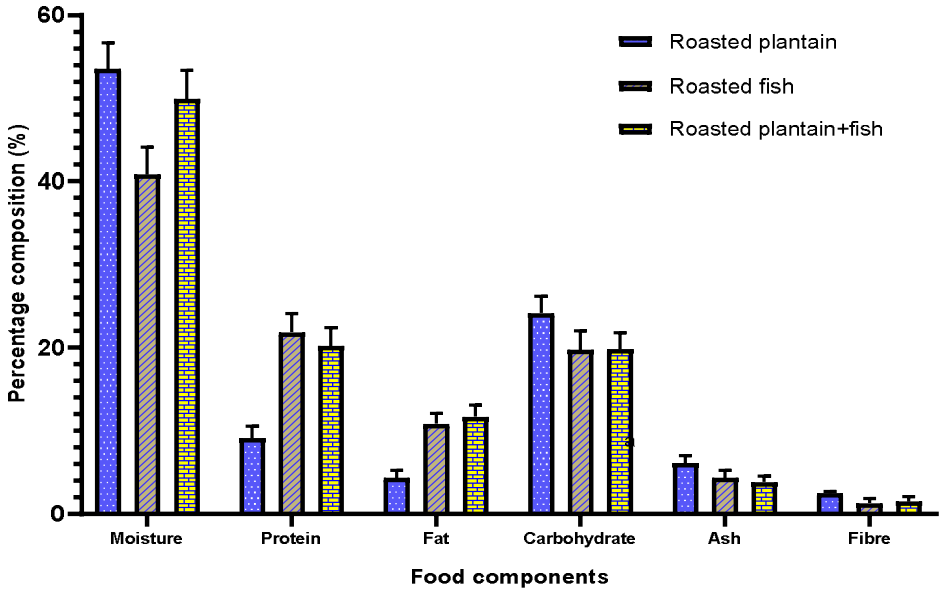

Figure 1: Proximate Composition of Roasted Plantain, Fish, and Plantain + Fish Sold in Otuoke Metropolis. Data are mean ± SD of triplicate determinations.

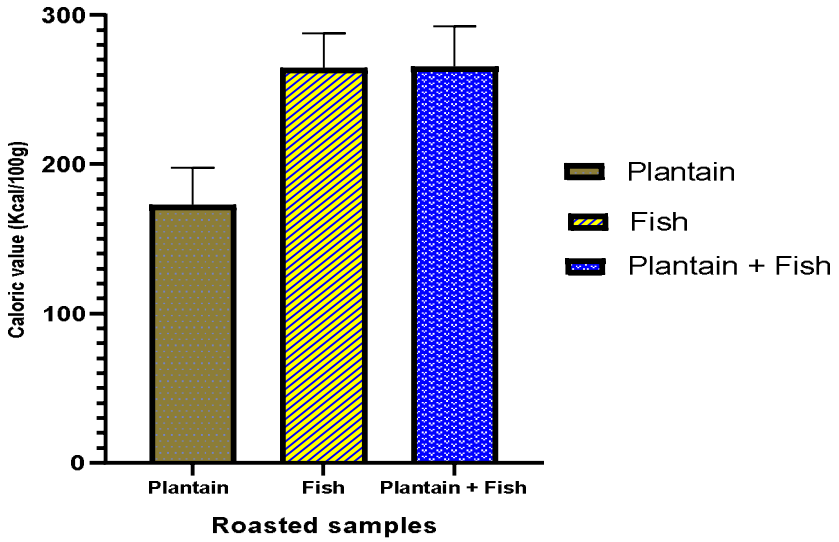

Figure 2: Caloric value of Roasted Plantain, Fish, and Plantain + Fish Sold in Otuoke Metropolis. Data are mean ± SD of triplicate determinations.

Proximate Composition and Caloric Value of Roasted Plantain and Fish

Proximate analysis of food is an integral and essential component of food studies, it gives a comprehensive insight into food nutrient components. These components include moisture, protein, fat, carbohydrates, ash and fibre. Information on these food components plays significant roles in nutritional appraisal, food quality hegemony, food processing and formulation and economic evaluation in guaranteeing nutritional sufficiency in our homes, regulatory conformity, and product development in both food and feed industries. The moisture content in food can considerably influence the appearance, texture, taste, shape, and weight of food products. It may also have implications for legal and labelling requirements, economically important requirements, the shelf life of the food or food products, food quality measurements, and food processing operations. The moisture content of the roasted samples, plantain (53.53 ± 3.13), fish (40.82 ± 3.35) and the combination of plantain and fish (42.91 ± 3.42) as presented in Figure 1.0 showed that they all have high moisture content. Foods with moisture contents of 40-55% are largely more vulnerable to microbial growth causing deterioration and spoilage and by extension diminished shelf life and repudiation of their safety [18, 19]. Furthermore, moisture content impacts food texture, weight, and appearance, which are essential for consumer acceptance and pricing in the market. High moisture foods therefore need cautious storage and handling to ensure food safety and quality, roasted plantain and fish are not meant to be kept outside the conditions for their preservation. Proximate analysis of the sample showed that the proportion of protein is in the order of $fish > plantain\ and\ fish > plantain.$ Proteins perform key biochemical functions acting as building blocks for cells, tissues and organs, serving as structural support, enzymatic processes, transport and storage, signaling and regulatory function, muscle contraction and movement, pH and fluid balance. they are also responsible for the biosynthesis of hormones, enzymes, blood plasma and immune promoters. The proximate composition ranges of protein from 9.14 ± 1.42 to 21.88 ± 2.22 are considered relatively high, with numerous nutritional and health benefits [20]. The proximate composition of crude fat in the sample showed that plantain is 4.39 ± 0.89, fish 10.87 ± 1.26 and the combination of plantain and fish 11.71 ± 1.43. these values are within modest fat concentrations that can furnish indispensable nutrients and energy, boost metabolic activities, and stimulate general wellness as a component of a balanced diet [20]. Carbohydrates often tagged as the "staff of life" are basically primary metabolites crucial for survival, biochemical sources of energy and precursors for the majority of secondary metabolites [21]. They can undergo hydrolysis to yield a six-carbon monomeric unit (glucose) which is routinely exploited through the

glycolytic pathway for energy generation or stored as glycogen in the muscles and liver for future use [22]. The proximate composition of carbohydrates in the sample under investigation showed that plantain is 24.18 ± 2.04, fish 19.75 ± 2.29 and the combination of plantain and fish 19.80 ± 1.99, these values are quite significant and can assist both metabolic and physiological functions [23].Ash embodies the aggregate mineral content in food, which can suggest the existence of essential nutrients and contribute to food's shelf-life and stability. Foods with ash content within the range found in the samples as plantain 6.12 ± 0.90, fish 4.39 ± 0.89 and the combination recipe of plantain and fish 3.86 ± 0.75 are mostly more nutrient-dense and can contain essential minerals like calcium, potassium, and magnesium, which are advantageous for health and vitality [24]. Fibre intake has been reported Fuller *et al.*[25] to support digestive health by stimulating routine bowel movements and inhibiting constipation, helping in gut microbiome health. Additionally, fibre aids the control of blood sugar levels by reducing glucose absorption, which helps manage and prevent type 2 diabetes, reduces the risk of cardiovascular disease and some types of cancer, predominantly colorectal cancer. The concentrations of fibre detected in the sample are sufficiently moderate to confer the above stated benefits

Findings from the study showed that the roasted plantain has a caloric value of 172.79 ± 25.12, fish with caloric value of 264.35 ± 23.41 and the combination of the plantain and fish has a caloric value of 265.39± 27.11. The caloric value of the roasted plantain reflects reasonably low energy density, ideal for individuals with weight management goals or those with lower caloric needs with respect to physical activity. In contrast, that of the fish and the combination of plantain and fish is considered a high caloric value and provides more energy per serving and maybe suitable for persons with higher caloric demands or those in need of quick energy like athletes while also contributing to weight gain and related health concerns if the diet is deficient in balance and variability [20].

Effect of Compounded Diet of Roasted Plantain and Fish on Body Weight/Organ Weight Ratio

The effect of the compounded diet of roasted plantain and fish on body weight/organ weight ratio in Wistar rats is presented in Figure 3.

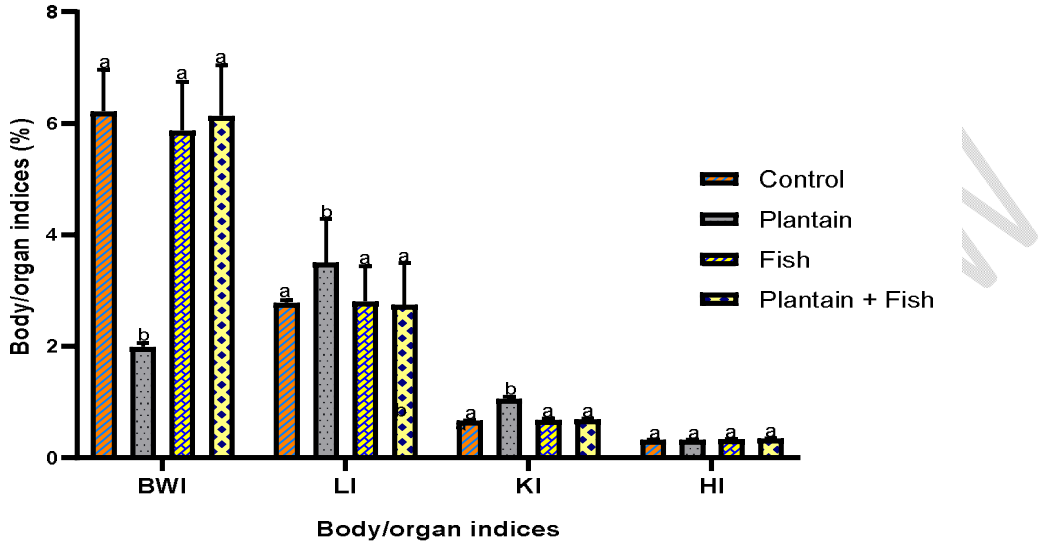

Figure 3: Effect of Compounded Diet of Roasted Plantain, Fish, and Plantain + Fish Sold in Otuoke Metropolis on Body Weight and Organ/Body Weight Ratio in Wistar Rats.

Data are mean ± SD (n = 5), values in the same row with different superscript letters are significantly different $p < 0.05$ while values with the same superscript letters are not significantly different $p > 0.05$. One-way Analysis of Variance (ANOVA), posthoc-tukey.

Key: BWI- body weight increase; LI- liver indices; KI- kidney indices; HI- heart indices

Body weight indices are significant pointers to the general health status of an individual [26], it is also a potent cursor to the harmful effects of xenobiotics.Kpomah*et al.*[27] posited that an estimated 15-29% loss in body weight in 5-7 days is critical. Evaluation of organ weight is also considered a delicate measure of xenobiotic toxicity. This is usually established as significant differences in organ weights between the treated and the control group, occurring with a lack of morphological alterations [28]. Therefore, the evaluation of changes in organ weight in the presence of body weight differences can result in the use of an organ-to-body weight ratio to investigate the toxicity of xenobiotics (Michael *et al.*, 2007). Findings from the study showed that there are non-significant differences ($p > 0.05$) in the growth rate between the control and all the other groups except the roasted plantain-fed group ($p < 0.05$). There was also a non-significant difference ($p > 0.05$) between the control and other groups with respect to liver, kidney and heart ratio or indices except the roasted plantain-fed group. Physical observation showed that the rats were not properly disposed to the

consumption of the roasted plantain diet, which also indicates subacute adverse effects on the growth rate and organ ratio of the roasted plantain-fed group.

Weekly Cage Side Observation for Physical Signs of Toxicity of Compounded Diet Made from Roasted Plantain and FishRecipe Sold in Otuoke Metropolis on Male Wistar Rats

Table 1 presents the findings of the weekly cage-side observation of the respective groups for overt signs of toxicity. None of the groups experienced mortality; in like manner, all groups did not show overt signs of toxicity except the roasted plantain-fed group, which presented on days 7 and 14 signs of restlessness, piloerection, and loss of appetite.

Table 1: Weekly Cage Side Observation for Physical Signs of Toxicity of Compounded DietMade from Roasted Plantain and Fish Sold in Otuoke Metropolis on Male Wistar Rats

S/N	PHYSICAL SIGNS OF TOXICITY	DAY 1				DAY 7				DAY 14			
		Control	Plantain	Fish	Plantain + Fish	Control	Plantain	Fish	Plantain + Fish	Control	Plantain	Fish	Plantain + Fish
1	Salivation	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Lacrimation	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Eye dullness	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Eye opacity	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
6	Restlessness	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Yes	Nil	Nil
7	Red stained muzzle	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
8	Lethargy	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Yes	Nil	Nil
9	Piloerection	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Yes	Nil	Nil
10	Skin appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Subcutaneous swelling	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
12	Loss of appetite	Nil	Nil	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Yes	Nil	Nil
13	Colour and consistency of faeces	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Abdominal distension	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
15	Mortality	0	0	0	0	0	0	0	0	0	0	0	0

Subacute Effects of Compounded Diet made from Roasted Plantain and Fish Sold in Otuoke on some Haematological Parameters of Male Wistar Rats

The subacute effects of a compounded diet made from roasted plantain and fish sold in Otuoke on some haematological parameters of male Wistar ratsare presented in Table .

Table 2:Subacute effects of compounded diet made from roasted plantain and fish sold in Otuoke on some haematological parameters of male wistar rats

Haematological Parameters	EXPERIMENTAL GROUPS			
	Control	Plantain only	Fish only	Plantain + Fish
WBC ($\times 10^3 UL^{-1}$)	8.72 ± 1.41^a	10.94 ± 1.83^b	9.01 ± 1.55^a	8.70 ± 1.64^a
RBC ($\times 10^3 UL^{-1}$)	6.35 ± 1.12^a	5.01 ± 1.00^b	6.22 ± 1.20^a	6.40 ± 1.22^a
PCV (%)	43.50 ± 3.25^a	38.82 ± 3.16^b	43.41 ± 3.53^a	45.70 ± 3.42^c
Hb (g/dL)	11.80 ± 1.62^a	9.29 ± 1.56^b	11.42 ± 1.81^a	12.20 ± 1.95^a
MCV (fl)	68.70 ± 4.40^a	59.89 ± 4.58^b	67.98 ± 3.68^a	70.00 ± 4.73^a
MCH (pg)	29.84 ± 2.20^a	24.10 ± 2.13^b	29.90 ± 1.38^a	29.75 ± 2.35^a
MCHC (gdL^{-1})	40.35 ± 3.15^a	35.11 ± 3.11^b	41.01 ± 3.27^a	41.00 ± 3.16^a
Platelet ($\times 10^9 L^{-1}$)	8933.12 ± 20.50^a	8421.11 ± 19.24^b	8900.22 ± 22.23^a	8900.00 ± 21.89^a

Data are mean ± SD of triplicate determinations, values in the same row with different superscript letters are significantly different $p < 0.05$ while values with the same superscript letters are not significantly different $p > 0.05$. One-way Analysis of Variance (ANOVA), posthoc-tukey.

KEY: WBC-White blood cell, RBC-Red blood cell, PCV-Packed cell volume, Hb-Haemoglobin, MCV-Mean corpuscular volume, MCH-Mean corpuscular haemoglobin, MCHC-Mean corpuscular haemoglobin concentration.

Haemoglobin, as the most frequently used biomarker of anaemia, plays an important function in the perfusion of bodily tissue. Red blood cell (RBC) count reveals the number of circulating RBCs and is essentially beneficial in categorizing erythrocytosis. A waning in RBC count, haemoglobin, and/or the blood levels of MCH, MCV, and MCHC is a symptom of anaemia[29] and cardiovascular disease [30]. Platelets help conserve the integrity of the vascular tree and yield platelet plugs in the first stage of clotting and platelet factor 3, a necessary factor of the coagulation cascade. Platelets are swiftly moved to injured tissues or sites of infection and possibly regulate inflammatory processes by networking with leukocytes, secreting cytokines, chemokines, and other inflammatory mediators. Platelets are of great importance in the initiation of thrombosis; therefore, their morphological and functional changes are closely correlated with the occurrence and development of coronary

artery thrombosis. Platelet count or monitoring is a significant factor in disease diagnosis as it gives intuition into various health conditions, predominantly those linked to clotting disorders, immune dysfunction, and haematologic disorders [31]. Additionally, a rise or reduction in the count is a pointer to disorders in hemostasis or liver disease, leukaemia and other types of cancer [32]. White blood cells (WBCs) are cellular components in humoral and cell-mediated immunity. Subclasses of WBC, such as neutrophils, lymphocytes, monocytes, or ratio of neutrophil to lymphocyte counts (N/L), are considered veritable and reliable predictive biomarkers to assess host immunity [33, 34].

Findings from this study showed that all groups had no significant change ($p > 0.05$) in all haematological parameters except the roasted plantain-fed group. An indication that the roasted fish, and the combinations of the roasted plantain and fish had no subacute adverse effect on the haematological parameters investigated.

Subacute Effects of Compounded Diet made from Roasted Plantain and Fish Recipe Sold in Otuoke on some Plasma Enzyme Activity of Male Wistar Rats

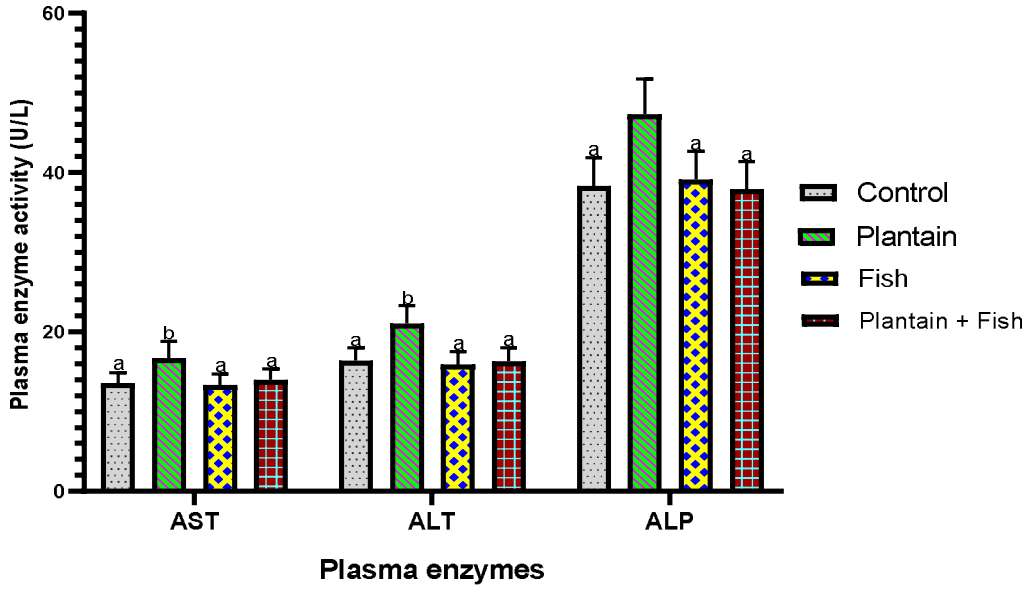

Figure 4: Effect of the compounded diet of roasted plantain, fish, and plantain + fish sold in Otuoke on some plasma enzyme activity of wistar rats.

Data are mean ± SD (n = 5), values in the same column with different superscript letters are significantly different $p < 0.05$ from the control group. One-way Analysis of Variance (ANOVA), posthoc-tukey.

Key: AST-Aspartate aminotransferase, ALT-Alanine aminotransferase, ALP-Alkaline phosphatase

AST occurs in high concentrations in the liver, kidney, heart, skeletal muscle tissues and erythrocytes. Injury to these tissues increases the AST concentration in the blood [35]. ALT

is located ubiquitously in the kidney, heart, skeletal muscle, brain, pancreas, spleen, and lungs. Specifically, the highest tissue concentration of ALT can be seen in the cytosol of hepatocytes. It catalyses the transfer of amino groups from the L-alanine to alpha-ketoglutarate, and the reaction products are L-glutamate and pyruvate which is vital in the tricarboxylic acid cycle. Pyruvate is used in the citric acid cycle to generate cellular energy. The release of ALT from injured hepatocytes causes increased serum ALT concentrations, and thus, it is more explicit for hepatic damage [35]. ALP is an abundant membrane-bound glycoprotein that catalyzes the hydrolysis of phosphate monoesters. ALP is a vital biomarker, and increases in serum concentrationcorrelate with bone and liver diseases [36]. Decreased concentration of ALP is usually rare compared to its increase, although it may occur in conditions such as hypophosphatasia, postmenopausal women on estrogen therapy, men with fresh cardiac surgery, malnutrition, magnesium deficiency, hypothyroidism, severe anaemia, and children with achondroplasia [36].

Findings from the study revealed that there was no significant difference $(p > 0.05)$ between the control and other groups with regards to the levels of AST, ALT and ALP except for the roasted plantain-fed group.An indication that all groups had no subacute adverse effect on these serum enzyme activities except the roasted plantain-fed group

Subacute Effects of Compounded Diet Made from Roasted Plantain and Fish Recipe Sold in Otuoke on *In-vivo* Antioxidant Status of Male Wistar Rats

The subacute effects of the compounded diet made from roastedplantain and fish Recipe sold in Otuoke on the *in-vivo*antioxidant status of male wistar rats is presented in Figure 5

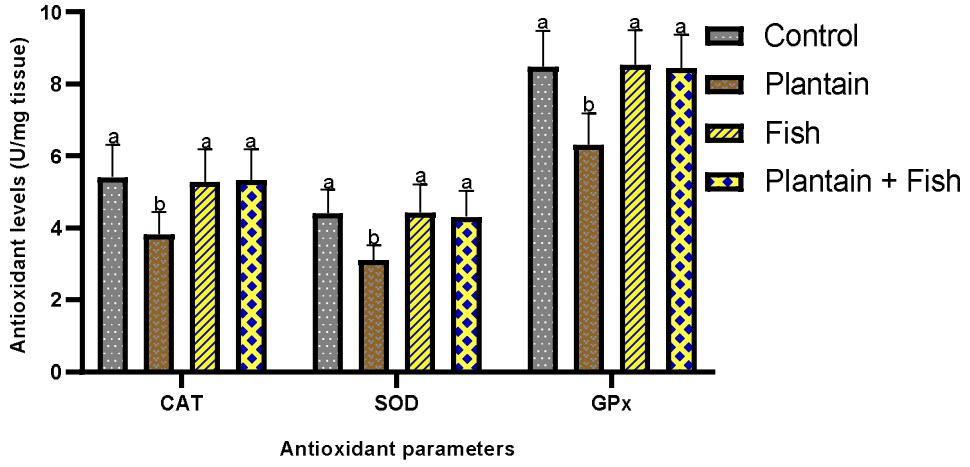

Figure 5:Subacute effects of compounded diet made from roastedplantain and fishsold in Otuoke on *in-vivo*antioxidant status of male wistar rats

Data are mean ± SD (n = 5), values in the same column with different superscript letters are significantly different $p < 0.05$ from the control group. One-way Analysis of Variance (ANOVA), posthoc-tukey.
KEY: CAT-Catalase, SOD- Superoxide dismutase, Gpx- Glutathione Peroxidase activity.

Oxidative stress is simply an imbalance between reactive oxygen species (ROS) formation and its detoxification thereby promoting an increase in ROS levels leading. ROS induces injury to cellular components leading to lipid peroxidation, nucleic acid, and protein modifications. The formation of lipid peroxidation and the subsequent alteration of nucleic acid and protein are primary etiological factors in the initiation and progression of various metabolic and neurodegenerative diseases [9]. SOD is crucial in protecting cells from cellular damage by catalysing the dismutation of superoxide radicals into oxygen and hydrogen peroxide, further which other antioxidants such as catalase and glutathione peroxidase can further detoxify [37]. This process is vital in preserving redox homeostasis and ameliorating oxidative stress, a primary factor in the aetiology of aging and various diseases. Catalase is a key enzyme that plays a criticalrole in protecting cells from oxidative damage by breaking down hydrogen peroxide, a toxic derivedfrom several metabolic processes into water and oxygen. This reaction is key in preventing oxidative damage to cellular components such as DNA, proteins, and lipids [38]. Glutathione peroxidase (GPx)plays an important role in

clinical chemistry due to its antioxidant effects and its participation in reducing oxidative stress within cells. GPx enzymes reduce hydrogen peroxide and organic hydroperoxides to water and alcohol respectively, with the aid of glutathione as a substrate.GPx concentrations are of clinical concern in conditions like ferroptosis, cardiovascular diseases, diabetes, and cancer [39, 40].

The finding from the study indicated that there were non-significant differences ($p > 0.05$) between the control group and all other groups with respect to CAT, SOD and GPx except the roasted plantain-fed group. This is an indication of non-adverse subacute effect with regard to the antioxidant status of the rats. The serum enzyme activities in the roasted plantain fed group, however, significantly reduced ($p < 0.05$) these antioxidant enzymes.

Subacute Effects of Compounded Diet Made from Roasted Plantain and Fish Recipe Sold in Otuoke Malondialdehyde Concentrations of Male Wistar Rats

The subacute effects of compounded diet made from roasted plantain and fish recipe Sold in Otuoke on malondialdehyde concentrations of male wistar rats are presented in Figure 6

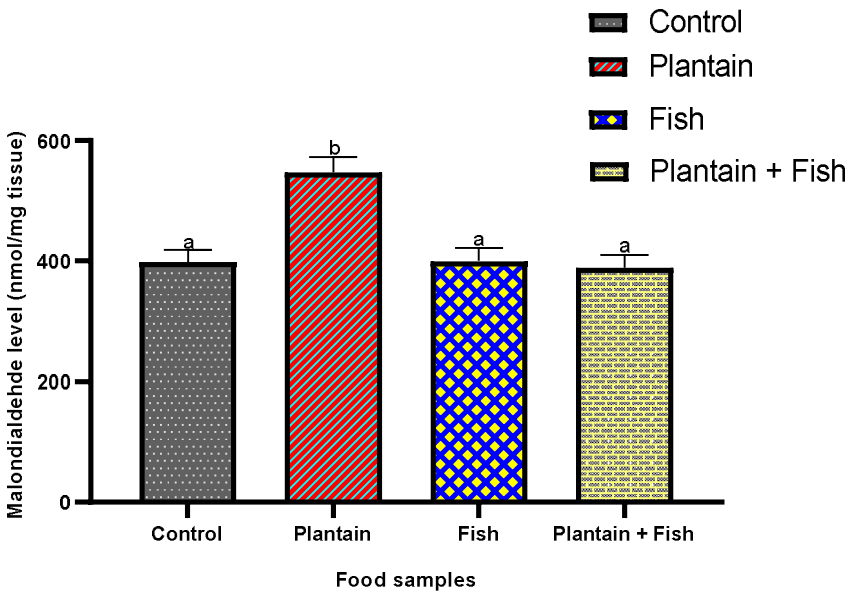

Figure 6: Subacute effects of compounded diet made from roasted plantain and fish recipe sold in Otuoke on malondialdehyde concentration of male wistarrats

Data are mean ± SD (n =5), values in the same column with different superscript letters are significantly different $p < 0.05$ from the control group. One-way Analysis of Variance (ANOVA), posthoc-tukey.

Malondialdehyde (MDA) is an important biomarker in clinical chemistry, it is extensively used to evaluate oxidative stress arising from lipid peroxidation. Its role is indispensable in fathoming innumerable disease mechanisms associated with oxidative damage. Higher than normal levels of MDA can imply increase oxidative stress, which is associated with inflammatory conditions such as cardiovascular disease, diabetes, and rheumatoid arthritis[41, 42].

Data from this study indicated that lipid peroxidation as depicted by malondialdehyde (MDA)concentration was not significantly different $(p > 0.05)$ between the control group and all other groups except the roasted plantain fed group which had MDA significantly increased $(p < 0.05)$ compared to the control group.

Conclusion

The subacute toxicity study showed that only the roasted plantain-fed group had significantly reduced body weight gain, and increased liver and kidney to body weight ratio ($p < 0.05$). The plasma enzyme activity (AST, ALT and ALP) of the plantain-fed group were also significantly increased ($p < 0.05$). The antioxidant status (CAT, SOD and GPx),haematological parameters (RBC, PCV, Hb, MCV, MCH, MCHC and platelet) of the plantain-fed groups were also significantly decreased ($p < 0.05$), while other groups had non-significant changes ($p > 0.05$) when compared to the control group. Malondialdehyde levels in the barbecued plantain-fed group were also significantly increased ($p < 0.05$).

Ethical Approval

Ethical clearance for this study and the use of experimental animals was approved by the Directorate of Research and Quality Assurance, Federal University Otuoke, Bayelsa State vied an authorization memo DRQA/FUO/ 0100/13/05/24.

Disclaimer (Artificial Intelligence)

Author(s) with this declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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