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Research Paper

Ameliorative Effect of Periwinkle Diet on Lipid Profile and Some Biochemical Parameters in Phenylhydrazine-induced Derangements

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ABSTRACT

Background: Anaemia is a haematological anomaly characterized by the deficiency of Red blood cell (RBC) count, Haemoglobin content, and Packed cell volume (PVC). It is a serious global health concern that particularly affects young children and pregnant women; WHO estimates that 42% of children less than 5 years of age and 40% of pregnant women worldwide are anaemic. Periwinkles are water-bodies resources of substantial economic value in the world of fisheries. They form a vital source of protein and are deposited in different water bodies. Seafoods are abundant sources of selenium, Vit A, Vit D, Vit E, Vit B12, Vit B6, thiamine, proteins, and essential fatty acids. They have also been shown to enhance erythropoiesis.

The present study aimed to investigate the biochemical boosting capacity of *T. fuscatus* diet ([TFD], having high antioxidants, vitamins, and mineral content) and other associated parameters on *Wistar* rats following Phenylhydrazine (PHZ)-induced hemolytic anemia and possible mechanism of action.

Methods: A total of 24 male *Wistar* rats with average weights of 180-250g were used for this study. The rats were randomly assigned into four groups (n=6). Group 1 (Control) received normal feed and drinking water. Group 2 received 600 mg/kg body weight of TFD orally. Group 3 received PHZ at a dose of 40 mg/kg body weight intraperitoneally. Group 4 received the same as Group 2 (600 mg/kg body weight of TFD orally) plus Group 3 (40mg/kg body weight of PHZ, intraperitoneally). The research lasted 42 days; thereafter, the rats were sacrificed, and blood samples were collected for relevant analysis.

Results: The result indicates an increase ($P<0.05$) in low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), cardiac risk ratio, atherogenic coefficient, atherogenic index in plasma, malondialdehyde, alanine transaminase, alkaline phosphatase, aspartate transaminase, Na^+, Cl^-, total bilirubin, unconjugated bilirubin, urea, uric acid, and creatinine in PHZ group compared to control and TFD group but were reversed i.e. decreased ($P<0.05$) in PHZ+TFD group compared to PHZ group. High-density lipoprotein-cholesterol (HDL-C), Catalase, Superoxide dismutase, Glutathione peroxidase, Total protein, Albumin, Globulin, K^+, HCO_3^-, Fe^{2+}, Ca^{2+}, and CB were all decreased ($P<0.05$) in PHZ group compared to control and TFD; however, they were all increased ($P<0.05$) in the PHZ+TFD group compared to PHZ.

Conclusion: PHZ-induction in *Wistar* rats is associated with atherogenic and hepatoxic impairment. The TFD diet ingestion is capable of ameliorating this condition via modulation of pro/antioxidant activities.

Keywords: Anemia, Periwinkle, Phenylhydrazine, Seafood, *Tympanotonos fuscatus*

Introduction

Seafoods fall among the category of useful forms of aquatic creatures nature has blessed us with; they form a vital source of protein and are deposited in different water bodies [1]. They vary in forms: roe, fish, and shellfish; the shellfish is composed of crustaceans, echinoderms, and mollusks. Periwinkles are classified into the mollusk family and are a crucial dietary nutrient source [1].

Periwinkles are water-bodies resources of substantial economic value in the world of fisheries. Bivalves and cephalopods contribute to the majority of molluscan fishery, with gastropods contributing less than 2% of the total production [2], although some gastropods are of relatively high economic value [3]. As man's population increases, the need for global fisheries products, including mollusks, rises yearly, leading to the exploitation of new stocks. The West African Mud Creeper, *Tympanotonos fuscatus* (T.F), is a univalve gastropod of the Phylum Mollusca and the only existing species in the genus *Tympanotonos* [4]. Its shell is much harder and stronger than that of a typical

snail's shell. It is a prosobranch-type of periwinkle commonly found in many brackish water creeks, estuaries, and mangrove swamps within Lagoons in Nigeria [5]. Periwinkles are unique and important marine invertebrates that are used as dietary supplements. One relevant factor among others that influences shellfish consumption is the quality of the meats [2]. *T. fuscatus* meat has been used traditionally as a subsistence food source with extremely nutritious quality for culinary delicacy.

Periwinkles have become vital in the preparation of some Nigerian delicacies like Okoro, Afang, and Edikang-Ikong soups, as well as Ekpang. This tasty mollusk has many health benefits and is sumptuous to behold. Nigerians have started using periwinkle as a commercial meat, though the utilization is yet limited, with little scientific information regarding the nutritional status of the gastropod [6].

Tympanotonos fuscatus contains important nutritive substances, such as vitamins, minerals, and significant amounts of fat called omega-3 fatty acids, known to lower cholesterol levels in the blood, which is important in reducing the incidence of coronary heart disease [7]. In addition, it contains a significant amount of cholesterol, iron, copper, Phosphorus, magnesium, and zinc [8]. It has also been reported that seafood is an abundant source of selenium, Vit A, Vit D, Vit E, Vit B12, Vit B6, thiamine, proteins, and essential fatty acids [9]. Additionally, they have been shown to enhance erythropoiesis [10]. Periwinkles are vital iron derivatives [6,8], which are necessary for the production and synthesis of hemoglobin, a major component of red blood cells (RBC).

Blood is a connective tissue that has arisen due to the evolutional trend of requiring a fluid medium for the function of the cells. It circulates through the heart, arteries, veins, and capillaries [11] and is considered the fluid of life, health, and growth [12].

Blood is made up of plasma, the liquid portion of blood, and cellular elements: RBC, white blood cells (WBCs), and platelets. However, blood transports nutrients and respiratory gases to vital organs; hormones and enzymes to target areas, waste products to excretory organs, and regulates acid-base balance and water [17].

Anaemia is a haematological anomaly characterized by the deficiency of RBC count, Haemoglobin content, and Packed cell volume (PVC). It is a serious global health concern that particularly affects young children and pregnant women; WHO estimates that 42% of children less than 5 years of age and 40% of pregnant women worldwide are anaemic [13]. Generally, reduction in RBC count, haemoglobin content, and PCV occurs because of declined production of RBC, increased rupture of RBC, and haemorrhage; all these events could either occur as inherited disorders or environmentally-induced scenarios, such as nutritional problems, infection, and exposure to drugs or toxins [14]. Symptoms may include but are not limited to fatigue, shortness of breath, dizziness, feeling cold, headache, sore tongue, pale skin [15].

Materials and Methods

Preparation of T. *fuscatus* Diet (TFD)

Fresh Periwinkle was purchased from Watt Market Calabar, Cross River State (Calabar, Nigeria); it was screened to remove debris, stone and sands. After immersing whole periwinkle in tap water in a 1:1 (w/w) ratio and boiling it for 30 min until the flesh pops up, the edible portion was removed, minced, and filtered through a mesh filter. *Tympanotonus fuscatus* diet (TFD) was prepared according to the method reported by [16]. The filtrate was oven-dried to powdered form to be used as TFD and was stored at -2ºC until use. TFD treatment was performed orally by packing this powdered form in a gelatin capsule (size 9el, Torpac Inc., USA); we placed the capsule in the stomach using a dosing syringe for a period of 6 weeks (42 days).

Experimental Animals

A total of 24 male *Wistar* rats weighing between 180-250g were used for this study. The animals were purchased from the Department of Physiology, University of Calabar (Nigeria) and kept under hygienic and favorable conditions (ventilated cages at room temperature of 27°C and exposed to a normal 12/12 hours light/dark cycle) in the animal house of the faculty. The animals were acclimatized for one week before the experimentation. The rats were allowed unrestricted access to pelletized feed and clean water.

Experimental Design

The rats were randomly assigned into four groups (n=6) as follows:

Group 1 (Control) received normal feed and drinking water

Group 2 received 600 mg/kg body weight of TFD orally

Group 3 received Phenylhydrazine (PHZ) at a dose of 40 mg/kg body weight intraperitoneally

Group 4 received the same as Group 2 (600 mg/kg body weight of TFD orally) plus Group 3 (40mg/kg body weight of PHZ, intraperitoneally)

We administered PHZ according to a previous study by Archibong et al. [18], while TFD dosage was determined based on the outcome of the LD_{50} value, which was found to be above 5000 mg/kg body weight [18]. The TFD administration was performed orally by packing the powdered form in a gelatin capsule (size 9cl, Torpac Inc., USA); we placed the capsule in the stomach using a dosing syringe for 6 weeks (42 days).

Collection and Analysis of Blood Samples

Blood samples of the animals were obtained via

cardiac puncture and introduced into plain sample bottles to determine biochemical parameters.

Measurement of Serum Lipid

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein-cholesterol (HDL-C) were analyzed according to the kit method as described by Johnson et al. [19] and Hassarajani et al. [20]. Very low-density lipoprotein (vLDL) concentration was determined according to a standard method as used by Ernest Atelhe Amama et al. [21]. In contrast, low-density lipoprotein (LDL) concentrations were determined based on Friedewald formula [22] as follows:

LDL_c = Total Cholesterol-(HDL_c+$vLDL_c$)

Atherogenic indices were determined using different formulas; therefore, Cardiac Risk Ratio (CRR)=TC/HDL-C [23], Atherogenic coefficient (AC)=(TC–HDL-C)/HDL-C [24], and Atherogenic index of plasma (AIP)=log (TG/HDL-C) [24].

Assay of Serum Antioxidants Enzymes and Serum Proteins

Gamma Glutathione (GGT) and malondialdehyde (MDA) activities were determined using the method as described by [25,26,27] respectively. Catalase (CAT) activities were determined using the method described by Aebi [28], and the change in the absorbance was monitored spectrophotometrically at 240nm over a 5-minute period. Superoxide dismutase (SOD) activities in serum were determined by assessing the inhibition of pyrogallol-auto oxidation [29]. Changes in the absorbance at 40 nm were recorded at 1-min intervals for 5 min. SOD activity was determined from a standard curve of % inhibition of pyrogallol auto-oxidation with SOD activity [29]. Serum levels of albumin, globulin, and total protein were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits and an automatic biochemistry analyzer (Mindray BS-800, Shenzhen, China) as used by [30].

Assay of Liver enzymes

Alkaline phosphatase (ALP) was analyzed according to a standard method as described by Bowers Jr GN and McComb R. B [31] and used recently by Archibong Nsa Archibong et al. [32].

Aspartate transferase (AST) and alanine transferase (ALT) activities in serum were analyzed according to standard methods as described by Reitman S and Frankel S [33] and employed by Archibong Nsa Archibong et al. [32].

Measurement of Serum Electrolytes and Minerals

The sodium, potassium, and chloride concentrations were determined using an ion-selective electrolyte analyzer (Biolyte 2000/ BioCare Corporation, Hsinchu 300, Taiwan) [34]. The bicarbonate ion level was measured using a standard method described by Mohite Bhavna V [35].

Measurement of Biochemical Parameters

Serum concentrations of urea were determined using Berthelot's reaction as described by Kaplan, A., and Teng, L. L [36], while serum concentrations of creatinine were determined following the method of Estridge, B. H. et al. [37] using the Reflotron Dry Chemistry Analyzer. The bilirubin (Conjugated, Unconjugated, and total bilirubin) level was measured as described by Jendrassik, L., and Grof, P. [38] as employed by Archibong, A. N. et al. [39].

Statistical Analysis

Statistical analysis was performed using GraphPad Prism (version 8.0.2) software. The results are presented as mean±SEM. The normality of the data was tested using the Shapiro-Wilk test, while the homogeneity of variance was tested using Levene's test. After confirming the normality of the distribution and homogeneity of variance, one-way analysis of variance (ANOVA) was employed to analyze the data, and a post hoc test (Least Square Difference) was performed to make comparisons. It is noteworthy that the values of $P<0.05$ were considered significant.

Results

Effect of TFD on Lipid Profiles and Atherogenic Indices in PHZ–Induced Anemic Wister Rats

Markers of atherogenic indices were investigated, and the results revealed that Tc, TG, and LDL-c concentration in PHZ groups were significantly higher ($P<0.01$ and $P<0.05$) when compared with that of the control and TFD groups, respectively; however, these were reduced following treatment with TFD as shown in PHZ+TFD treated group. Conversely, the HDL-c concentration in the PHZ group was significantly lower ($P<0.01$) when compared with that of the control and PHZ+TFD treated group, respectively; however, this was significantly increased following treatment with TFD as shown in PHZ+TFD treated group. However, the difference in vLDL-c among the groups was of no statistical significance, as shown in Table 1. Other markers of atherogenic indices were measured; therefore, the CRR, AC, and AIP in the PHZ group were significantly higher ($P<0.05$ and $P<0.01$) when compared with that of control and TFD-treated groups. However, these were reduced following treatment with TFD, as indicated in PHZ+TFD treated groups (Table 2).

Table 1. Lipid profiles in different experimental groups

Group	TC	TG	HDL-c	LDL-c	VLDL-c
Control	2.90±0.19	0.22±0.02	1.07±0.02	2.05±0.09	0.12±0.02
TFD	2.60±0.21	0.22±0.02[c]	1.14±0.03[d]	1.80±0.19	0.12±0.02

PHZ	4.04±0.19**,a	0.31±0.02*	0.74±0.00**	2.95±0.08**,a	0.16±0.01
PHZ+TFD	3.56±0.17b	0.26±0.02	0.95±0.02	2.55±0.20b	0.14±0.02

Values are expressed as mean±SEM, *=P<0.05 & **=P<0.01 vs control, a=P<0.001 & b=P<0.05 vs TFD, c=P<0.01 & d=P<0.001 vs PHZ,

Table 2. Markers of atherogenic indices in different experimental groups

Group	CRR	AC	AIP
Control	9.71±0.84	8.71±0.84	0.02±0.05
TFD	7.62±0.58	6.62±0.58	0.01±0.04b
PHZ	12.1±0.89a	10.94±0.96a	0.2±0.02**
PHZ+TFD	1.063±1.21	9.63±1.21	0.09±0.02

Values are expressed as mean±SEM,**=P<0.01 vs control, a=P<0.05 vs TFD, b=P<0.001 vs TFD

Effect of TFD on Markers of Oxidative Stress: An assay of markers of oxidative stress revealed that CAT, SOD, and GPx activities in the PHZ groups were significantly lower (P<0.01 and P<0.05) when compared with that of the control and TFD group respectively. However, these significantly increased following treatment with TFD, as shown in the PHZ+TFD treated groups. Conversely, MDA activities in the PHZ group were observed to be significantly higher (P<0.05) when compared with that of the control and TFD groups; nevertheless, this was significantly reduced following treatment with TFD as shown in PHZ+TFD treated group (Table 3).

Effect of TFD on Total Protein Concentration: Total protein, albumin, and globulin concentrations in the PHZ groups were observed to be significantly lower (P<0.001 and P<0.01) when compared with those of the control and TFD groups, respectively; however, this was increased following treatment with TFD as shown in PHZ+TFD treated groups (Table 4).

Effect of TFD on Liver Enzymes Activities in PHZ–Induced Anemic Wister Rats

Assay on markers of liver functions revealed that ALT, ALP, and AST activities in the PHZ groups were significantly higher (P<0.01) when compared with those of the control and TFD-treated groups, respectively; nevertheless, these were significantly reduced following treatment with TFD as shown in PHZ+TFD treated groups (Table 5).

Table 3. Antioxidant and malondialdehyde activities in different experimental groups

Group	CAT	SOD	MDA	GPx
Control	0.37±0.01	0.22±0.02	12.17±0.07	976.5±20.21
Tf Diet	0.41±0.02	0.23±0.02	11.67±0.80c	1059±53.43
PHZ	0.27±0.03*,b	0.12±0.02**,a	16.00±0.63*	818.7±56.46**
PHZ+TFD	0.35± 0.03	0.16±0.01b	14.00±1.24	883.3±36.68b

Values are expressed as mean±SEM, *=P<0.05 & **=P<0.01 vs control, a=P<0.01 & b=P<0.05 TFD, c=P<0.05 vs PHZ

Table 4. Total protein, albumin and globulin concentration in different experimental groups

Group	Total protein	Albumin	Globulin
Control	66.33±0.80	34.33±0.62	32.67±0.42
TFD	65.83±0.54	36.00±0.73	32.33±0.56
PHZ	55±0.37***,a	24.00±0.73***,a	27.5±0.43***,a
PHZ+TFD	62.17±0.48**,b	28.00±1.16***,a	29.5±0.62**,a

Values are expressed as mean±SEM, ***=P<0.001 vs control, a=P<0.001 & b=P<0.01 vs TFD

Table 5. Liver enzymes activities in different experimental groups

Group	ALT	ALP	AST
Control	47.28±3.26	0.94±0.08	43.16±2.08
TFD	45.54±3.82a	0.89±0.06c	42.42±1.80b
PHZ	70.33±3.91***	1.69±0.14**	59.28±4.10**
PHZ+TFD	51.8±0.79b	1.08±0.07a	47.76±2.67c

Values are expressed as mean±SEM, ***=P<0.001 vs control, a=P<0.001, b=P<0.01 & c=P<0.05 vs PHZ

Effect of TFD on Electrolytes and Minerals Concentration in PHZ–induced Anemic Wister Rats

The investigated electrolyte concentration revealed that Na^+ and Cl^- concentrations in the PHZ groups were significantly higher (P<0.01) when compared with that of the control and TFD-treated groups, respectively. However, these were significantly reduced following treatment with TFD, as shown in PHZ+TFD treated groups. Conversely, the K^+ and HCO^- concentrations in the PHZ groups were significantly lower (P<0.05) when compared with those of the control and TFD groups, respectively; these were significantly increased following treatment with TFD as shown in PHZ+TFD treated groups (Table 6a).

The investigated minerals components revealed that the Fe^{2+} and Ca^{2+} concentrations in the PHZ groups were significantly lower (P< 0.01 and P<0.001) when compared with that of the control and TFD-treated groups; however, these were significantly increased following treatment with TFD as shown in PHZ+TFD treated groups (Table 6b).

Table 6a. Electrolyte concentration in different experimental groups

Group	Na^+	Cl^-	K^+	HCO_3
Control	139.7±0 .67	103.2±0.60	4.98±0.06	22.17±0.60
TFD	135.2±0.98*	102.2±0.60	5.40±0.13	24.00±0.68

PHZ	147.7±0.49***,b	111.7±1.12***,b	4.43±0.06*,b	18.5±0.76**,b
PHZ+TFD	141.8±0.62a,c	109±0.58***,b	4.85±0.06d	20.83±0.70d

Values are expressed as mean±SEM,*=P<0.05, **=P<0.01 & ***=P<0.001 vs control, a=P<0.01, b=P<0.001& d=P<0.05 vs TFD, c=P<0.001 vs PHZ

Table 6b. Minerals and erythropoietin concentration in different experimental groups

Groups	Fe	Ca
Control	467.3±1.52	2.40±0.06
TFD	503.2±0.95***	2.57±0.07
PHZ	379.5±1.23***,a	1.45±0.12***,a
PHZ+TFD	450.3±0.88***,b	1.75±0.15**,b

Values are expressed as mean±SEM, **=P<0.01 & ***=P<0.001 vs control, a & b=P<0.001 vs TFD

Discussion

Blood is a connective tissue in fluid form and the process of its formation is called hematopoiesis. This process occurs in the bone marrow, producing different forms, such as erythrocytes, leucocytes, and thrombocytes. Blood is known to perform many healthy functions [12,10]; however, these functions can be compromised by several factors, including damage to the bone marrow, kidney, and liver, including haemolytic anaemia. PHZ-induced hemolytic anaemia is associated with lipid peroxidation, free radicals, and reactive oxygen species (ROS) [40, 41], thereby causing derangement in erythropoiesis and erythrocyte functions [42, 17].

The present study aimed to investigate the biochemical boosting capacity of TFD (having high antioxidants, vitamins, and mineral content) and other associated parameters on *Wistar* rats following PHZ-induced hemolytic anemia and possible mechanism of action.

The present study demonstrates that TFD treatment had cardioprotective and anti-atherogenic effects. It was observed that PHZ induction brought about an increase in TC, TG, and LDLc concentrations when compared with those of the control groups. This result is an indication that PHZ induction is capable of causing lipid peroxidation, which may increase the chances of atherosclerosis [43, 44], which was reduced following TFD treatment. This result is important and may be attributed to active components in the TFD, such as vitamins (A, D, E, C, B1, B2, B3), oleic acid, and essential fatty acid (Omega-3). These dietary components are known to reduce the level of TC and LDL-cholesterol in the body [45].

It was also observed that PHZ induction brought about a decrease in HDL when compared with that of the control group. The PHZ induction can result in dyslipidemia, neurotoxicity, increase in the level of circulating cholesterol ester transfer protein (CETP) and lipoprotein-associated phospholipase A_2 (LP-LA_2), due to lipid peroxidation [46]. The reversal of this adverse effect following treatment with TFD could probably be due to Omega-3 fats capable of boosting HDL and lowering the cholesterol level in the system. The increase in HDLc in the TFD-treated group is important because it helps reduce the level of TC, thereby preventing the risk of atherosclerosis. These results were corroborated by other results, including those related to CRR, AC, and AIP, which all indicated the cardioprotective effect of TFD treatment.

The antioxidant enzyme activities were also investigated, and the results demonstrated that CAT, SOD, and Glutathione peroxidase (GPx) activities were significantly downregulated following PHZ induction when compared with those of the control group. Anaemia is a serious disease that affects health and its effect can be aggravated by increased oxidative stress and increased free radicals. Little wonder why the CAT, SOD, and GPx activities in this study were significantly downregulated following PHZ induction, and this is in line with Kendall [47]; however, these were all significantly increased following treatment with TFD. The TFD contains important vitamins, e.g., C, E, and A [48], which can serve as potent antioxidants, thereby increasing antioxidant activity. Therefore, TFD treatment may play an important role in reducing free radical's effect in anemic conditions. The MDA activity in the PHZ group was significantly increased compared to that of the control group. MDA is an important marker for oxidative stress and lipid peroxidation [49, 50]; its activity was significantly upregulated following PHZ induction, revealing to what extent ROS and free radicals were generated and how detrimental they could be to health. This result is consistent with Sundaram et al. [51]; however, this was significantly reduced following treatment with TFD, indicating its useful health benefit.

The biomarkers of Serum protein and liver function were also investigated; it was observed that the TP, albumin, and globulin concentrations were significantly reduced following the induction of PHZ; this result is significant because it is supported by El-Shafey *et al.* [52], which shows a drastic downregulation of serum protein concentration in anaemic condition. This deleterious effect was reversed following treatment with TFD. Increased globin concentration in plasma indicates immune system stimulation [53] since globulin is formed almost entirely in the lymphoid tissues and constitutes the antibodies that form the immune system. The elevated globulin also indicates that treatment with TFD can prevent microcytic hypochromic anaemia since globin is involved in iron transport in the blood. Moreover, the serum albumin results indicated that liver cells were not damaged since albumin concentration is decreased in both chronic and acute liver diseases [54].

The ALT, ALP, and AST activities were upregulated

following PHZ induction. This result is also supported by Obayuwana *et al*. [55], which shows the upregulation of liver enzyme activities following anaemic conditions; however, this was also reversed following treatment with TFD. Liver enzyme activities have been shown to be stabilized following treatment with TFD [18, 56]. This may be due to the presence of an active component of Omega-3 fatty acid known as hepatoprotective [57] since up-regulation of ALT and ALP are common in kidney, pancreatic, heart, and liver damage conditions.

In the present study, electrolyte concentration results indicated that PHZ induction was able to bring about a drastic increase in Na^+ and Cl^- concentration; this result is significant because elevated Na^+ concentration predisposes one to high blood pressure [11], which means that PHZ induction is capable of predisposing one to hypertension; however, this inappropriate trend was reversed following treatment with TFD. The reduction of sodium ion concentration following TFD treatment has been indicated in Akwari *et al.* [58]; this may be due to the low concentration of sodium in the diet or possibly due to the ability of the diet to potentiate excretion of sodium ions from the body. The Cl^- concentration also followed the same trend since sodium and chloride ions are always transported alongside [12].

It was also observed in the study that K^+ and HCO_3^- concentrations were reduced following PHZ induction but reversed following treatment with TFD. The increase in potassium ion concentration observed in the TFD-treated group may be brought about by the decrease in serum sodium ions occasioned by its excretion and reabsorption of potassium ions since sodium and potassium ions are always exchanged alternately by the Na^+/K^+ pump along the cell membrane [36]. Bicarbonate is produced by the pancreas and liver to neutralize the acidic pH produced by the acid in the gastrointestinal tract. Bicarbonate ions also maintain the acid-base buffering system of the blood. Bicarbonate ion concentration increased in serum following TFD treatment, indicating that it can promote good health. Induction of PHZ was shown to bring about a reduction in serum Ca^{2+} concentration, which was reversed after treatment with TFD. This result is significant because, generally, TFD is rich in Ca^{2+} [59]; therefore, elevated serum Ca^{2+} may be useful in preventing bone reabsorption and other related conditions associated with calcium deficiency.

The result of this study indicated that PHZ induction brought about an increase in the levels of organic compounds (urea and creatinine). Urea is the by-product of erythrocyte metabolism, while creatinine is one of the waste products in the blood. Serum urea and creatinine concentration were increased, as observed in the PHZ group, indicating renal disease [60]. The increased urea concentration following PHZ intoxication is consistent with Kale *et al.* [41] and also reflects the hemolytic destruction of erythrocytes observed in the PHZ group as blood urea concentration increases following erythrocyte destruction, which was reduced following the treatment with TFD. The TFD have been known to be rich in certain components, e.g., minerals (Fe^{2+}, Zn^{2+}, Cu^{2+}, Se) and vitamins (A, C, E), which are potent antioxidants [61]. They can boost the erythropoietic processes, thereby ameliorating the toxic effect induced by PHZ on erythrocytes, which is evidenced by the decreased concentration of organic compounds in the TFD-treated groups.

Conclusions

In conclusion, the present study indicates that PHZ-induction in *Wistar* rats is associated with atherogenic and hepatoxic impairment. The TFD ingestion can ameliorate this condition via modulation of pro/antioxidant activities.

Compliance with Ethical Guidelines

The rats were handled based on the 1985 guidelines of the National Institute of Health publication for laboratory animals. Ethical approval and guidelines on animal experiments were obtained from the University of Calabar Ethics Committee (Nigeria), and the approval number was 240PHY2723.

Authors' Contributions

ANA and SEO conceptualized the study. ANA, SEO, USU, and ISE designed the study. ANA, USU, EAO, and ISE contributed to the bench work. CCM, DUO, and OEO provided expert advice and knowledge. All authors contributed to the development of the final manuscript and approved its submission. ANA, SEO, and ISE prepared the first draft, which was reviewed by USU, EAO, DUO, OEO, and CCM.

Conflict of Interests

The authors declare that there is no conflict of interest.

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