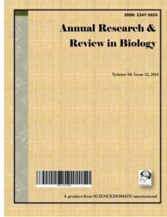

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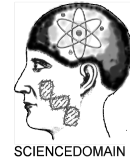

Biochemical Changes in Tissues of Wistar Albino Rats Following Exposure to Diet Incorporated with Locally Processed Fish

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Authors' contribution

This work was carried out in collaboration between both authors. Author LAN designed the study, wrote the protocol and interpreted the data. Author GOCO anchored the field study, gathered the initial data and performed preliminary data analysis while authors LAN and GOCO managed the literature searches and produced the finals draft. Both authors read and approved the final manuscript.

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ABSTRACT

Biochemical changes in tissues of Wistar albino rats following exposure to feed formulated with different percentage concentrations (5%, 10%, and 20%) of smoked fish after 28 days were investigated. Albino rats fed with animal feed without smoked fish served as the control. The concentrations of ascorbic acid, glutathione (GSH), total protein, albumin, globulin as well as the activities of catalase and lactate dehydrogenase (LDH) were determined/ assayed respectively, using standard methods. The results showed that there were no significant ($p>0.05$) differences in these parameters from rats fed with 5% smoked fish when compared to those of the control. However, there were a significant ($p<0.05$) decrease in the concentrations of ascorbic acid, glutathione, total protein, albumin, globulin and the activity of catalase in albino rats fed with feed formulated with 20% in contrast to increase in activity of lactate dehydrogenase in smoked fish-fed rats when compared to the control. The values obtained showed that, as the percentage of smoked fish in the formulated feed increased from 10%, the concentrations of ascorbic, glutathione and

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total protein (albumin and globulin) decreased. The catalase activity decreased in contrast to lactate dehydrogenase whose activity increased, indicating that the changes were concentration-dependent. The results obtained might in part indicate that the local process of smoking of fish, which involved high temperature and smoke could have induced synthesis of harmful substances probably [polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs)] in the smoked fish. These harmful substances might have caused the observed changes in the concentrations and activities of these oxidative stress parameters in the rats.

Keywords: Albino rats; smoked fish; oxidative stress parameters; polycyclic aromatic hydrocarbons; heterocyclic aromatic amines.

1. INTRODUCTION

Food processing steps such as roasting/ smoking, grilling and barbecuing generate polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs), while only very low levels of PAHs are identified when food is cooked by other cooking steps such as steaming [1]. Food processing at high temperatures (grilling, roasting, frying) are major sources of generating PAHs and HAAs. Polycyclic aromatic hydrocarbon and heterocyclic aromatic amine (HAA) are pro-carcinogens which can be bioactivated to carcinogens in the body by cytochrome P_{450} isoforms CYP1A/A2 and CYP2E1 respectively [1]. Studies have shown that heat processed meat at high temperatures can generate these genotic substances (PAH and HAA) [2]. PAHs can also enter the environment through natural sources such as oil seeps, forest fires and through a variety of anthropogenic activities. These include the burning of fossil fuel and wood, smelting of metals, petroleum refining, gas flaring and crude oil spill [3].

Food roasting/smoking belongs to one of the oldest technologies of food preservation which mankind has used in fish processing. Smoking has become a means of offering diversified, high value added products as an additional marketing option for certain fish species where fresh consumption becomes limited [4]. Traditional roasting/smoking techniques involve treating of pre-salted, whole or filleted fish with wood smoke in which smoke from incomplete wood burning comes into direct contact with the product; this can lead to contamination if the process is not adequately controlled [5].

Roasting/smoking of foods especially protein and fat-containing foods with intense heat over a direct flame results in fats dripping on hot fire yielding flames and smoke that contains PAHs and HAAs [6]. These substances are formed when fish or meat is smoked at a very high temperature (100℃-200℃) and a longer duration [7]. HAA synthesized when creatine is converted to creatinine which undergoes reaction with amino acids like phenyalanine, threonine or alanine to form HAAs. Benzo (a) pyrene [B (a) P] is recognized as a marker of PAH contamination [8]. Benzo (a) pyrene toxicity occurs by indirect attack on DNA, through the formation of a reactive epoxide: 9, 10 epoxide (benzo(a)pyrene-r-7, t-8-dihydrodiol–t-9,10-epoxide(BPDE) that damages cellular macromolecules such as proteins, lipids and DNA [9-12[.

Fish is an important food mostly eaten by virtually everybody because of its nutritionally significant role in supplying proteins, lipids, vitamins and important minerals. The aim of this study was to investigate the effect of frequent consumption of smoked fish on oxidative stress parameters.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty eight (28) male albino rats of the Wistar strain weighing between 156 to 186 grams were purchased from the animal house of the Department of Zoology, University of Nigeria Nsukka, Nigeria. The rats were transported to the animal house of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. The rats were housed in partitioned wire-meshed cages under standard laboratory condition of humidity, temperature (25±2℃) and light (12 hr light/dark cycles). They were treated humanely as encapsulated in National Institute of Health guidelines [13]. They were supplied with feed and water *ad libitum*.

2.2 Animal Feed

The animals were fed with (Growers pelletized feed) which was purchased from the animal

house of the Department of Zoology, University of Nigeria, Nsukka.

2.3 Animal Material

The fish used for this study was fresh Tilapia (*Tilapia mosambicus*), which was purchased from Obinze market, Owerri, Nigeria. The fishes were smoked using fire wood through direct flame for about four hours. They were homogenized with a manual mechanical grinder and stored in an air-tight container at room temperature prior to feed formulation. The feed was mixed with homogenized fish by percentage weight by weight according to the animal groups.

2.4 Experimental Designs

The rats were divided into four groups of seven (7) rats in each cage according to their relative body weights. The animals were allowed to acclimatize to the environment for one (1) week on a regular feed. After acclimatization, each group was fed with the feed formulated with 5%, 10%, and 20% w/w of smoked fish, except the control group which received 100% feed. During this period, observations were made on the animals' appetite and general wellbeing.

2.5 Animal Grouping and Feed Administration

The four (4) different experimental groups received designated concentrations of the feed mixed with smoked fish as follows:

> Group 1 received 100% feed (Control), Group 2 received 95% feed with 5% smoked fish, Group 3 received 90% feed with 10% smoked fish while Group 4 received 80% feed with 20% smoked fish respectively.

2.6 Collection of Blood Samples

The rats were anesthetized by exposure to dichloromethane vapour in covered transparent plastic container. Incisions were then made into their thoracic regions and were terminally bleed by cardiac puncture. The blood samples were collected using 5 mL hypodermic syringes and needles and introduced into sterile sample bottles. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10 mins. The serum was separated using micropipettes and used for the determination/assay of the various parameters.

2.7 Estimation of Oxidative Stress Parameters

Ascorbic acid concentration was determined by the method of Reo and Kuether [14]. Glutathione (GSH) concentration was determined by the method of Jallow et al. [15]. The method is based on the formation of a relatively stable chromophoric product on reacting with a suphurhydryl compound (GSH) with Ellman's reagent. Total protein concentration was determined by the methods of Tietz [16], while albumin and globulin concentrations were determined by the methods of Doumas et al. [17].

Catalase activity (CAT, E.C. 1. 11.1 1.) was assayed by measuring spectrophotometrically at 570 nm the rate of decomposition of hydrogen peroxide (H_2O_2) over a period of 30 minutes at (1 minutes interval) as described by Sinha [18]. The enzyme activity was expressed in terms of Katalase feiahigkeit (Kat.f) as $ks^{-1}\ mg^{-1}$ protein where K is the first order rate constant. Lactate dehydrogenase (LDH, E. C. 1.1.1.27) activity was assayed following standard procedures as described in the assay kit from Randox Laboratories Ltd, United Kingdom.

2.8 Statistical Analysis

Each reading was taken in triplicate. All data were expressed as mean ± standard deviation and analyzed for statistical significance by using one way Analysis of Variance (ANOVA). Differences in means were considered significance at $p \leq 0.05$.

3. RESULTS

The results of the concentrations of ascorbic acid (mg/ml) of rats fed with feed formulated with 5%, 10% and 20% smoked fish and control are presented in Fig. 1. The results show that there was no significant ($p>0.05$) difference in the concentrations of ascorbic acid of rats fed with feed formulated with 5% smoked fish when compared to those of the control. Smoked/ roasted fish significantly ($p<0.05$) decreased the mean concentrations of ascorbic acid from (13.20±2.70) in the control, (12.50±1.44) in rats fed with 5%, (8.52±1.20) in rats fed 10% and (4.22±1.50) in rats fed 20% smoked formulated feed respectively.

The results of the concentrations of glutathione (mg/ml) of rats fed with feed formulated with 5%, 10% and 20% smoked fish and control are presented in Fig. 2.

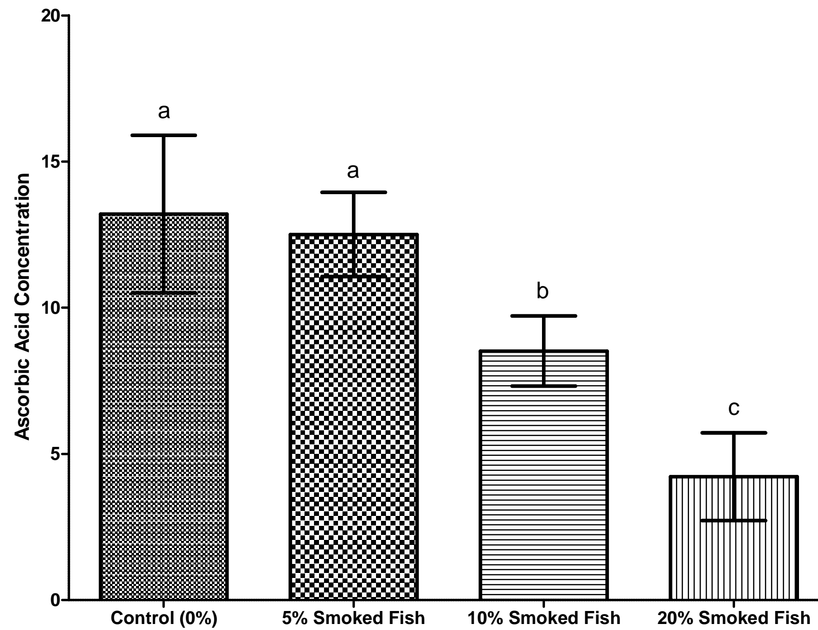

Fig. 1. Concentrations of ascorbic acid from rats fed with different percentage concentrations of smoked fish

**Bars with different superscript letters are significantly different at p<0.05*

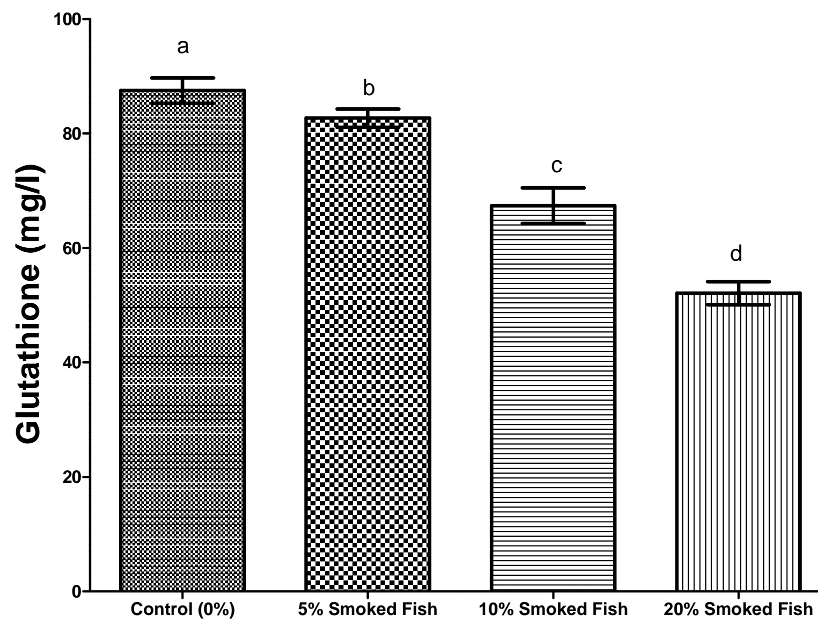

Fig. 2. Concentrations of glutathione from rats fed with different percentage concentrations of smoked fish

**Bars with different superscript letters are significantly different at p<0.05*

The results also show that there was no significant (p>0.05) difference in the concentrations of glutathione of rats fed with feed formulated with 5% smoked fish when compared

to the control. However, smoked fish significantly ($p<0.05$) decreased the mean concentrations of glutathione from (87.50±2.20) in the control, (82.71±1.60) in rats fed with 5%, (67.42±3.10) in rats fed 10% to (52.10±2.00) in rats fed 20% smoked formulated feed respectively.

Fig. 3 shows that there was no significant ($p>0.05$) difference in the serum total protein (albumin and globulin) of rats fed with feed formulated with 5% smoked fish when compared to the values obtained for the control. However, smoked / roasted fish significantly ($p<0.05$) reduced the mean concentrations of total protein, albumin as well as globulin (Fig. 3) in the rats fed with feed formulated with 10% and 20% smoked fish when compared to the control.

The results obtained for catalase activities (Kat.f) from this study are presented in Fig. 4. The results obtained from rats fed with feed formulated with 5% and the control followed the same trend as those for ascorbic acid and glutathione. But smoked/roasted fish significantly ($p<0.05$) decreased the mean activities of catalase from (7.30±1.00) in the control, (6.50±0.50) in rats fed with 5%, (4.40±0.10) in rats fed 10% to (2.70±0.08) in rats fed 20% smoked fish formulated feed respectively.

The results obtained for lactate dehydrogenase activity (U/l) from this study are presented in Fig. 5. The results obtained for rats fed with feed formulated with 5% and the control followed the same trend as those for glutathione and catalase but in the opposite direction. However, smoked/ roasted fish significantly ($p<0.05$) increased the mean activities of lactate dehydrogenase from (53.50±3.00) in the control, (60.20±4.00) in rats fed with 5%, (117.25±10.00) in rats fed 10% to (215.50±17.10) in rats fed 20% formulated feed respectively.

4. DISCUSSION

Our results revealed marked changes in all the parameters investigated (concentrations of ascorbic acid, glutathione, total protein, albumin and globulin; activities of catalase and lactate dehydrogenase in the serum of albino rats) in response to exposure to these sources of petroleum hydrocarbon (PAHs and HAAs) from 10% and 20% smoked-formulated feed (Figs. 1-5).

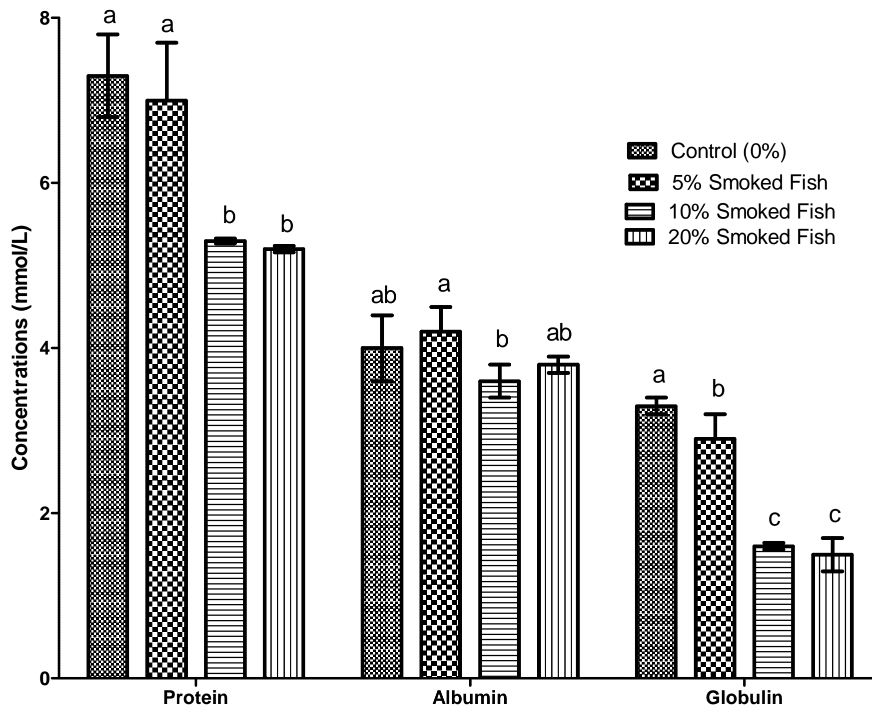

Fig. 3. Concentrations of serum protein, albumin and globulin from rats fed with different percentage concentrations of smoked fish

**Bars with different superscript letters are significantly different at p<0.05*

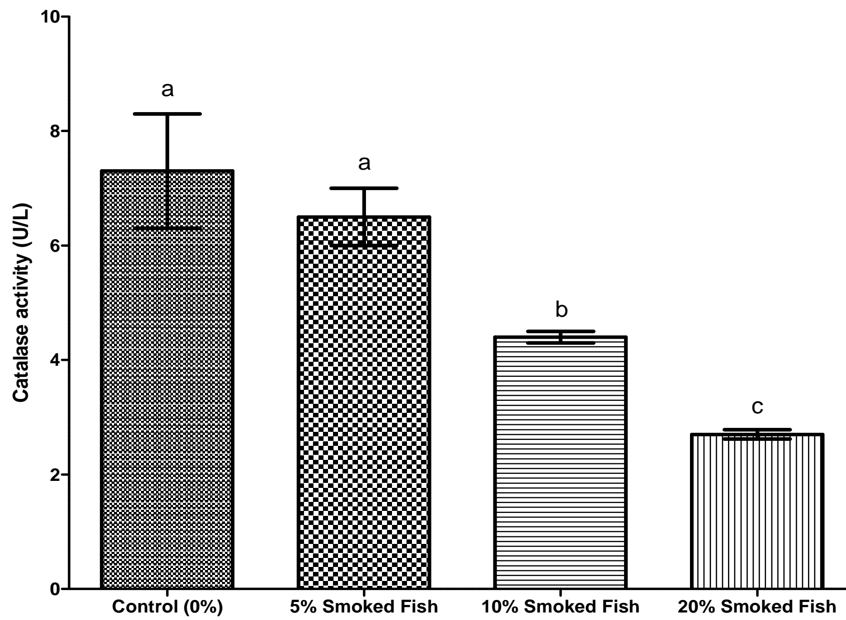

Fig. 4. Catalase activities from rats fed with different percentage concentrations of smoked fish

**Bars with different superscript letters are significantly different at p<0.05*

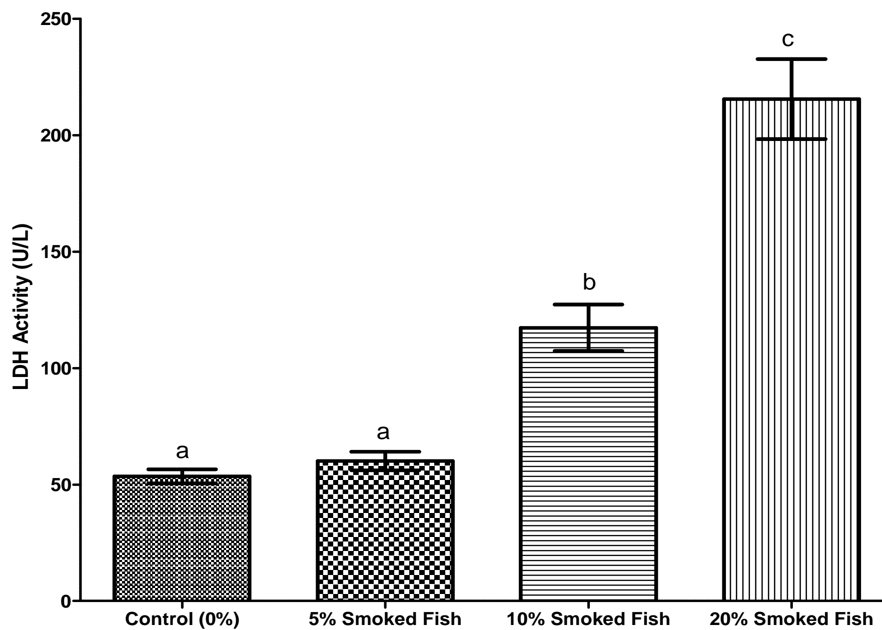

Fig. 5. Lactate dehydrogenase activities from rats fed with different percentage concentrations of smoked fish

**Bars with different superscript letters are significantly different at p<0.05*

Ascorbic acid is a water-soluble antioxidant found in the cytoplasm of cells, which scavenges free radicals in the cyosol [19]. Living organisms exposed to PAHs, HAAs and petroleum hydrocarbons produce a lot of free radicals which cause chain reactions of oxidations in living

organisms [20]. As a water-soluble antioxidant, ascorbic acid in conjunction with glutathione, vitamin E, lipoic acid and some enzymes such as catalase, glutathione peroxidase, superoxide dismutase help to quench free radical chain reactions that lead to oxidative stress, thereby causing changes in their concentrations and activities respectively in a living system [21].

The mean concentration of glutathione in the rats fed with 10% and 20% feed formulated with smoked/roasted fish was found to be significantly ($p>0.05$) lower than the value obtained for the control (Fig. 2). This could also be as a result of the antioxidant function of glutathione by which it scavenges free radicals induced by the PAHs and HAAs in the smoked fish. This is in accordance with the report of [22], that glutathione is associated with stress resistance owing to its redox-thiol group. Nwaogu et al. [21] also reported a reduction in the mean concentration of glutathione in the native fowl (*Gallus domesticus*) following chronic exposure to petroleum hydrocarbon pollution, although, the organisms used were not the same.

Living organisms exposed to an environment where there is high generation of free radicals as a result of pollution usually experience reactions such as oxidative protein modification among others which may result to cellular damage, if left unchecked by antioxidants. This may in part be explained by the significant ($p<0.05$) decrease observed in the concentrations of ascorbic acid, glutathione, total protein and the activity of catalase. This finding corroborates with the reports of [2].

Catalase is an enzyme found in nearly all living organisms (plants and animals) that are exposed to oxygen where it functions to catalyse the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen [23]. A concentration-dependent reduction was observed in the activities of catalase of rats fed with 10% and 20% of smoked fish-formulated feed when compared to those of the control. This finding also agrees with the reports of Ujownudu et al. [2] who worked on the hepatoprotective effects of crude extracts of tomato and onion in rats exposed to locally processed beef.

The mean activity of lactate dehydrogenase was found to be markedly higher in the rats fed with 20% followed by those fed with 10% smoked fish formulated feed when compared to those of the control. This result agrees with the reports of Stephen-Onodjede [24] who noted an increase in LDH activity of fowls native to Warri in Niger Delta with chronic PHC pollution when compared to that of fowls from Ughara, an unpolluted environment.

5. CONCLUSION

This study has shown that smoked fish formulated feed at high concentration induced reactive intermediates in rats which could cause reduction in antioxidants and consequently lead to debiliting effects to the living system. These changes if unchecked could precipitate various disease conditions especially oxidative stress related diseases.

ETHICAL APPROVAL

We, hereby declare that this study was approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria and all experiments were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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