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RESEARCH PAPER

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Changes in Kidney Function Profile of Wistar Albino Rats Fed with Diet-Containing Locally Processed Fish

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ABSTRACT

Changes in kidney function profile of Wistar albino rats fed with diet-containing different concentrations (5%, 10% and 20%) of locally processed fish for 28 days were investigated. The group fed normal animal feed served as the control. Serum concentrations of Na^+, K^+, Cl^- and HCO^-_3 ions, creatinine, urea and total bilirubin were determined using standard methods. Results obtained for serum electrolytes, creatinine, urea and total bilirubin of rats fed with 5% of smoked fish in diet indicated that there were no significant ($p>0.05$) changes in these parameters when compared with those of the control. However, significant ($p<0.05$) changes were observed in all the parameters in rats fed with 10% or 20% smoked fish when compared with those of the control. The values obtained showed that, as the percentage of smoked fish increased from 10%, the concentrations of serum electrolytes (Na^+, K^+, Cl^-, and HCO^-_3), decreased, indicating that the decrease was concentration-dependent in contrast to the concentrations of creatinine, urea and total bilirubin which increased. The results obtained might, in part, indicate that the local process of preparation of the fish, which involved high temperature and smoke could have induced the synthesis of polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs) in the roasted/smoked fish. These substances might have caused the observed changes in the kidney function profiles.

Key words: Albino Rats, Smoked Fish, Kidney Function Profile, Polycyclic Aromatic Hydrocarbons and Heterocyclic Aromatic Amines.

INTRODUCTION

Food processing methods for fish and meat such as roasting/smoking, grilling, barbecuing generate polycyclic aromatic hydrocarbons (PAHs) and heterocyclic aromatic amines (HAAs). Food processing at high temperatures (grilling, roasting/smoking and frying) is a major source generating PAHs and HCAs. Polycyclic aromatic hydrocarbons and heterocyclic aromatic amines are pro-carcinogens which can be bioactivated to carcinogens in the body by cytochrome P_{450} isoforms CYP1A/A2 and CYP2E1 respectively Felton et al., 2002). Studies have shown that heat-processed meat at high temperatures can generate these genotic substances or toxicants (PAH and HAA) Ujowundu et al., 2014). PAHs can also enter the environment through natural sources such as oil seeps and forest fires and through a variety of anthropogenic activities (Douben et al., 2003). These include the burning of fossil fuel and wood, smelting of metals, petroleum refining, gas flaring and crude oil spill (Douben et al., 2003).

Food roasting/smoking belongs to one of the oldest technologies of food preservation which mankind has used for fish processing. Smoking has become a means of providing diversified, high-value-added products as an additional marketing option for certain fish species Gomez et al., 2009). 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 (Gomez-Esta et al., 2011).

Roasting/smoking fish, meat or other foods with intense heat over a direct flame results in fats dripping on hot fire and yielding flames and smoke containing a number of PAHs and HAAs (Tzankis et al., 2001). These chemicals are formed when fish or meat is roasted/smoked at a very high temperature ($100^{o}C$-$200^{o}C$); and a longer duration can increase the PAH and HAA concentration especially in fatty and protein foods respectively (Larson et al., 1983). The synthesis of HAA is favoured in fish, meat and other protein-containing food materials. Here 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 (Swartz and Millison, 1985). Benzo(a)pyrene toxicity occurs by indirect attack on DNA, through the formation of a reactive epoxides: 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 (Dipple, 1984;Annas et al., 2002;Zhang et al., 1998; Zue and Warschawsky, 2005).

Fish is an important food eaten by virtually everybody because of its nutritionally significant role in supplying protein, lipids, vitamins and important minerals. The aim of this study was to investigate the effect of frequent consumption of roasted/smoked fish on kidney function profile and protein status of albino rats.

MATERIALS AND METHODS

Experimental Animals

Twenty eight (28) male albino rats of the Wistar strain weighing between 156 and 186 grams were purchased from the animal house of the Zoology Department, 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± 2^0C) and light (12 hr light/dark cycles). They were treated humanely as encapsulated in National Institutes of Health (NIH, 1995). guidelines. The research was approved by the Departmental Ethical Committee on the use of animals for the research, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. They were supplied with feed and water *ad libitum*.

Animal Feed

The animals were fed with (Growers pelletized feed) which was purchased from the animal house of the Zoology Department, University of Nigeria, Nsukka.

Animal Material

The fishes used were fresh *Tilapia mosambicus,* which was purchased from Obinze market, Owerri, Nigeria, and identified by a fish taxonomist at the Department of Fishery and Aquaculture, School of Agriculture, Federal University of Technology, Owerri. The fishes were smoked using fire wood through direct flame for about four hours and further dried for 48 hrs. They were homogenized with a manual mechanical grinder and stored in an air-tight container at room temperature prior to feed formulation.

Experimental Designs

The rats were divided into four groups of four (4) 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 which, each group was fed with the diet 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.

Animal Grouping and Feed Administration

The four (4) different experimental groups received designated concentrations of the feed mixed with smoked fish thus: Group 1 received100% 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

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 they were terminally bled by cardiac puncture. The blood samples were collected using 5mL hypodermic syringes and needles and introduced into plain sterile sample bottles without anticoagulant. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10mins. The serum was separated using micropipettes and used for the determination of the concentrations of the various parameters.

Biochemical studies

Serum sodium and potassium ion concentrations were determined by the ion selective electrode method using humalyte machine (Human, Germany) described by (Tietz, 1995). Serum chloride concentration was determined by the titremetric method of Schales and Schales (1941). Serum bicarbonate (as total CO_2) concentration was determined by the use of a CO_2, gas electrode (ASTRA CO_2 apparatus, Beckem Instruments, USA). Serum urea and creatinine concentrations were determined by Jaffe''s reaction and urease enzymatic method respectively described by Tietz, 1995). Serum total bilirubin concentration was determination according to Balistreri and Shaw (1987), using standard kits (Human Laboratories, Germany).

Statistical analysis

Each reading was taken in triplicate. All data were expressed as mean ± standard

deviation and analysed for statistical significance by using one way Analysis of Variance (ANOVA). Values were considered significant at p≤ 0.05.

RESULTS

The concentrations of Na^+, K^+, Cl^- and HCO^-_3 of rats fed with diet formulated with 5%, 10% or 20% smoked fish and those of control are presented in Table 1 and Figure 1. There were no significant (p>0.05) differences in mean serum electrolyte concentrations of Na^+, K^+, Cl^- and HCO^-_3 of rats fed with diet formulated with 5% fish when compared to those of the control. The results indicated that sodium ion concentrations for 5%, 10%, 20% and control ranged from 101.10± 0.6, 80.40±0.40 and 51.31± 0.83mmol/L and 121.89 ±0.37 mmol/L respectively. The same trends were observed for potassium, chloride and bicarbonate respectively. There was a significant decrease (p<0.05) in mean serum sodium, potassium, chloride and bicarbonate ion concentrations as the percentage of roasted fish of the formulated diet increased (Figure 1).

Table 1. The Concentrations of serum electrolytes, creatinine, urea and total bilirubin from rats fed with different percentages of Smoked fish in diet (mmol/L).

% Concentrations of smoked fish in diet.	Serum electrolytes concentration						
					Creatinine	Urea	Total bilirubin
	Na^+	K^+	Cl^-	HCO_3^-			
Control	121.58^a ± 0.37	5.15^a ± 0.50	115.10^a ± 04.40	20.23^a ± 01.32	0.27 ± 0.02	0.04 ± 0.02	0.02 ± 0.02
5	101.10^b ± 0.60	4.80^b ± 0.30	101.20^a ± 02.10	18.20^a ± 0.80	0.68 ± 0.03	0.42 ± 0.04	0.64 ± 0.03
10	80.40^c ± 0.40	3.10^c ± 0.10	98.30^a ± 03.30	13.50^b ± 0.90	0.78 ± 0.04	0.46 ± 0.03	0.74 ± 0.03
20	51.31^d ± 0.83	2.70^d ± 0.60	55.20^b ± 02.01	7.30^c ± 0.70	0.89 ± 0.03	0.64 ± 0.05	0.97 ± 0.01

***Values with different superscript letters are significantly different at P>0.05**

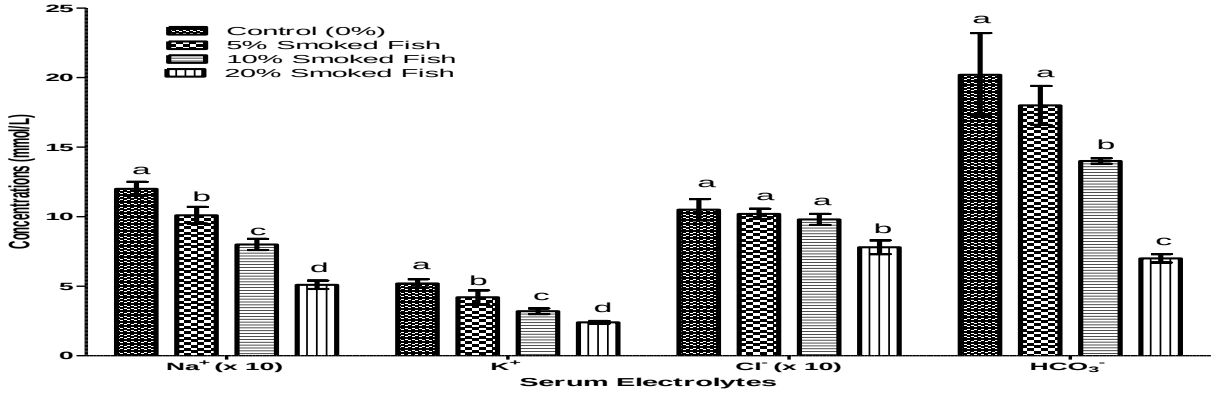

Fig. 1. Concentrations of serum electrolytes from rats fed with different percentage concentrations of smoked fish.

*Values with different superscript letters are significantly different at p<0.05.

The results of the concentrations of creatinine, urea and total bilirubin of rats fed with diet formulated with 5%, 10% or 20% smoked fish and those of control are presented in Table 1 and Figures 2-4. The results show that there was no significant ($p>0.05$) difference in serum concentrations of creatinine, urea or total bilirubin of rats fed with formulated with 5% smoked fish in diet when compared to those of the control. Smoked /roasted fish significantly ($p<0.05$) increased the mean concentrations of creatinine from (0.27±0.02 to 0.89±0.03), urea (0.40±0.02 to 0.71±0.05), and total bilirubin (0.30±0.01 to 0.97±0.03) mmol/L for the control and rats fed with feed formulated with 20% roasted fish respectively (Table 1, Figures 2-4).

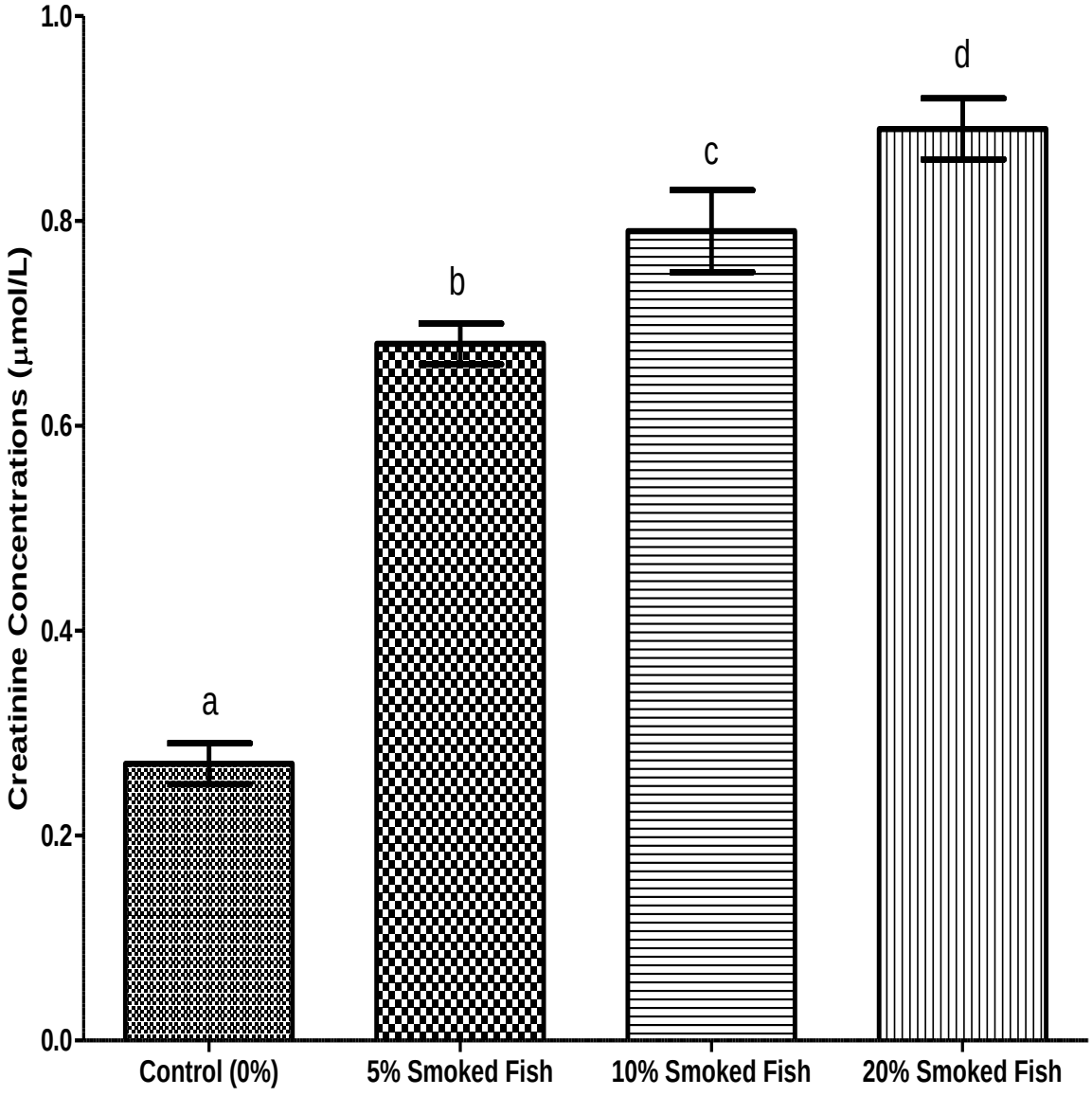

Fig. 2. Concentrations of creatinine from rats fed with different percentage concentrations of smoked fish.

*Values with different superscript letters are significantly different at $p<0.05$.

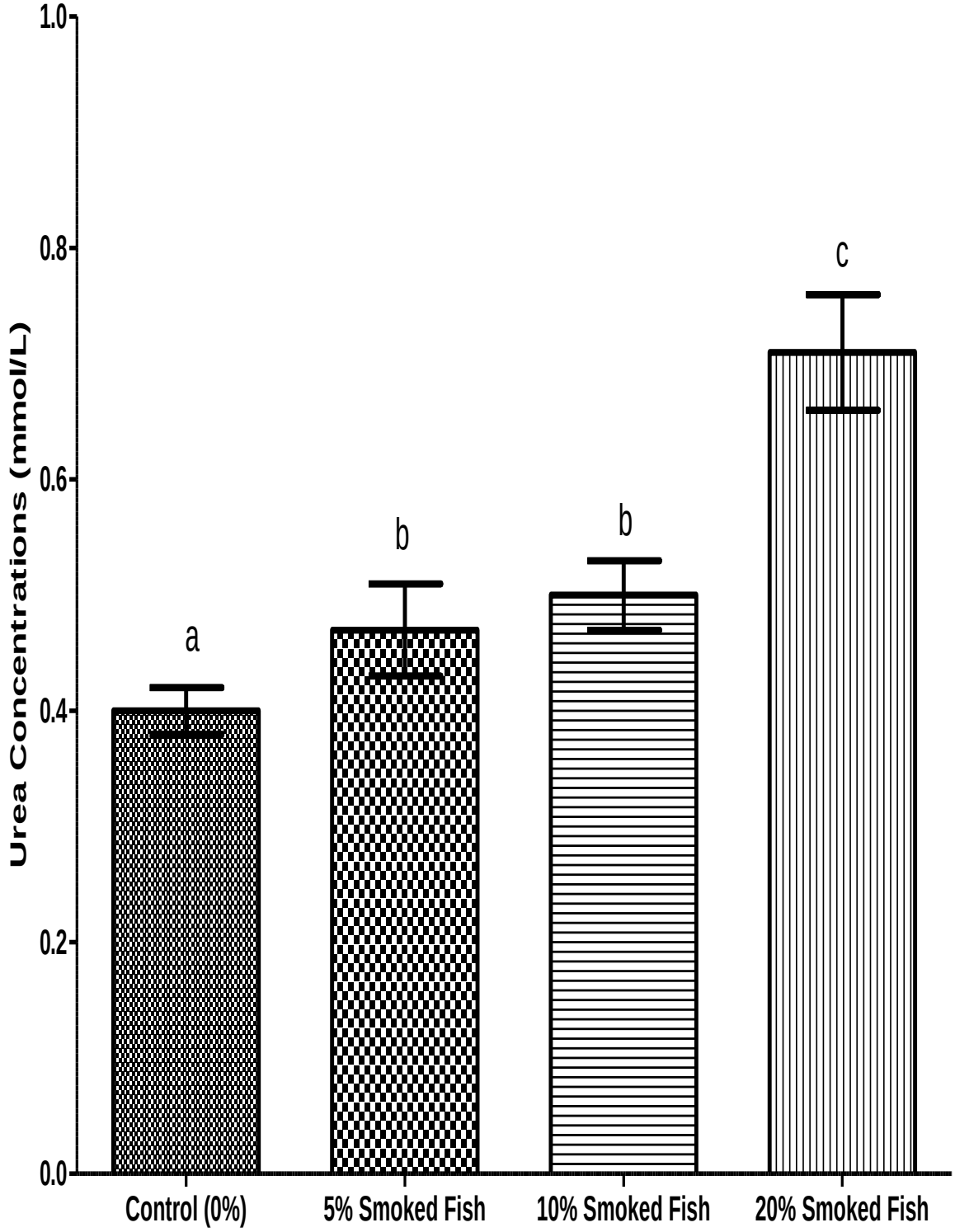

Fig. 3. Concentrations of urea from rats fed with different percentage concentrations of smoked fish.

*Values with different superscript letters are significantly different at $p<0.05$.

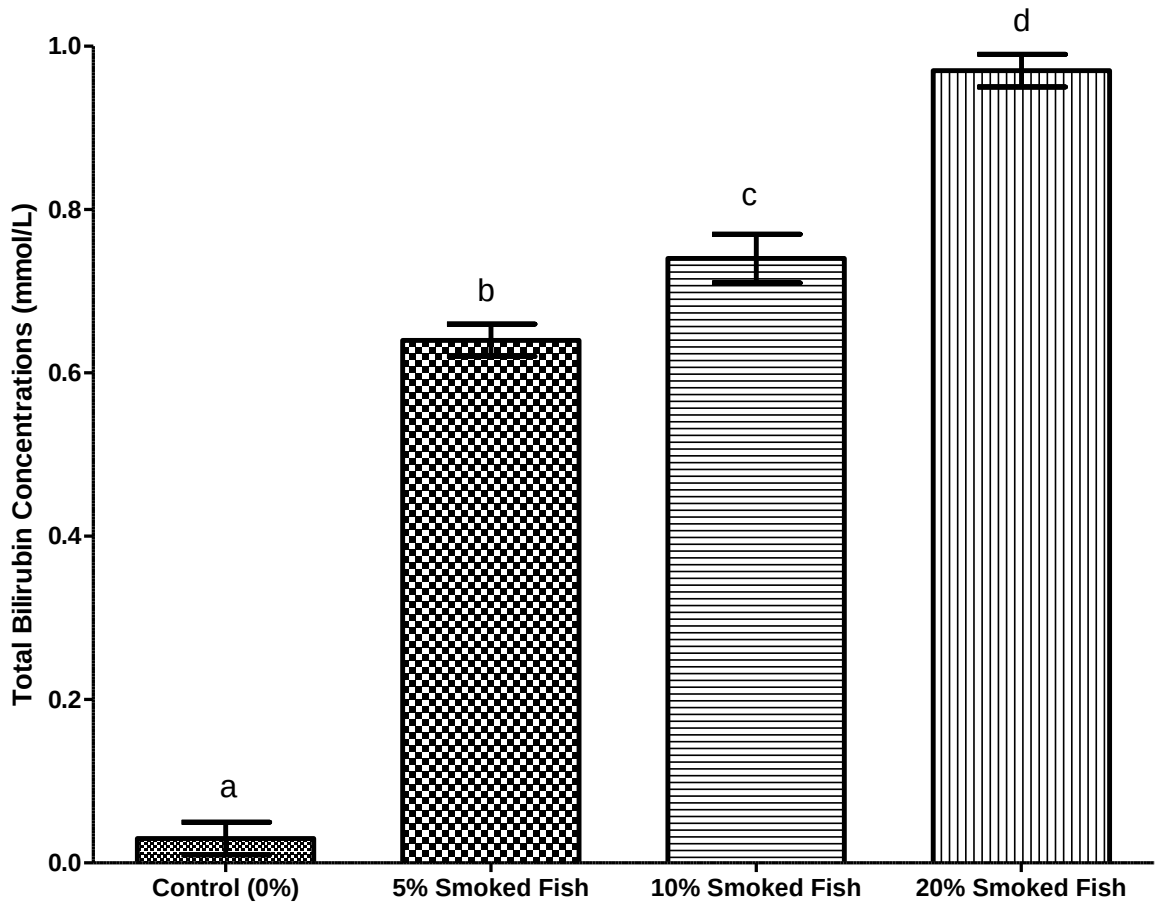

Fig. 4. Concentrations of total bilirubin from rats fed with different percentage concentrations of smoked fish.

*Values with different superscript letters are significantly different at $p<0.05$.

DISCUSSION

Studies have shown that the methods used in food preparation, such as roasting/smoking and barbecuing can increase the amount of synthesized PAH and HAA in foods (Knize et al., 1999). Food is said to contribute more than 90% of total exposure to PAHs (WHO, 1998). The formation of PAH and HAA on roasted/smoked foods has been observed to be dependent on the distance of food from the heat source and fat content of the food, duration of roasting and temperature used (WHO, 1998).

In this study, the results showed a significant decrease ($p<0.05$) in serum sodium ion concentration in rats fed the smoked fish diet when compared to those of the control. This decrease was dose dependent; as the percentage fish content of the formulated diet increased, the rat serum sodium ion concentration decreased. Sodium is an essential nutrient that regulates blood volume and blood pressure, maintains the right balance of fluids in the body, transmits nerve impulses, and influences the contraction and relaxation of muscles. It is also necessary for maintaining osmotic equilibrium and the acid-base balance. Sodium cation is important in neuron (brain and nerve) function, and in influencing osmotic balance between cells and the interstitial fluid, with their distribution mediated in all animals and in

some plants by the so-called Na^+/K^+ ATPase pump (Conwary and Clark, 1996).

Sodium makes up most of the cations of blood plasma and potassium makes up most of the cell fluid cations. Sodium and potassium are likely to be in the diet, are reabsorbed as the glomerulus of the kidneys carries out its filtration process. The significant decreases in the concentrations of sodium and potassium ions observed in this study might indicate that these elements were not adequately reabsorbed by the kidney. This situation could imply an organ malfunction probably as a result of the presence of PAHs and HAAs in the ingested diet. The re-absorption of sodium keeps the blood volume exactly right and the osmotic pressure correct while the re-absorption of potassium keeps the serum concentration as close as normal. Poor absorption of potassium for a time could result in hypokalemia and even death of the organism.

Significantly reduced values for serum bicarbonate were observed in rats fed varying amount of smoked fish in diet when compared to the values obtained for the control. However, a non-significant decrease was observed in serum bicarbonate in rats fed 5% smoked fish in diet. Bicarbonate is alkaline, and a vital component of the pH buffering system of mammals (maintaining acid-base homeostasis). This is especially important for protecting tissues of the central nervous system, when pH changes too far outside of the normal range in either direction could precipitate to health problems.

These results agree with earlier report of Hammad et al.,2013) who recorded a dose-dependent decrease in serum Na^+, K^+, Cl^- and HCO^-_3 concentrations in albino rats fed with different concentrations of acrylamide (Lehning et al.,1998). Lopachin *et al*, (1993) reported that acrylamide might cause distal anoxopathy by disrupting subaxonal distribution of Na^+, K^+ Cl^- and HCO^-_3.

The result obtained in this study is also in agreement with that of Orisakwe et al, (2004) who recorded a dose- dependent decrease in serum Na^+, K^+, Cl^- and HCO^-_3 concentrations of albino rats treated with Nigerian Bonny light crude oil which is known to contain polycyclic aromatic hydrocarbons.

Creatinine concentration reflects the glomerular filtration rate (GFR) .The GFR is clinically important because it is a measure of renal function (Harita et al., 2009). The effect of diet formulated with smoked fish on creatinine concentration of Wistar albino rats is presented in Table 1 and Figure 2. A rise in blood creatinine level is observed only with marked damage to functioning nephrons. This is as a result of renal dysfunction caused by the polycyclic aromatic hydrocarbon and hetero-cyclic amine known to be in the smoked/roasted fish. This study demonstrated this situation.

Total bilirubin is produced from the degradation of haemoglobin from erythrocytes undergoing normal (removal of aged cell) or abnormal destruction (ie intravascular or extravascular haemolysis) within mononuclear phagocytes (principally splenic, hepatic and bone marrow macrophage (Wakabayashi, 19990; Tomaro and Batlle, 2002).

Thus, PAH and HAA at high concentration might cause harmful effects on the kidneys which could lead to ineffective filtration at the glomerulus and/or damage to the tubular cells thereby reducing their ability to secrete or absorb vital substances including water (Tomaro and Batlle, 2002). Tomaro and Batlle (2002) reported that increased creatinine and urea concentrations above normal range indicate renal impairment associated with kidney function and

occasional nephritic syndrome. The kidney can suffer considerable damage before losing sufficient function to modify the normal clinical indication (Sood, 2009). The detection of renal damage at a reversible stage is necessary before effective preventive measures can be taken to halt the progress of damage to the irreversible stage. The significant ($p<0.05$) decrease in serum sodium, potassium, chloride and bicarbonate ion concentrations in the smoked fish treated groups when compared with those of the control is suggestive of renal pathology.

CONCLUSION

Considering the negative fluctuations of serum electrolytes (Na^+, K^+, Cl^- and HCO^-_3), creatinine, urea, total bilirubin, observed in this study, these findings are suggestive of possible alteration in renal function of these rats fed high percentage concentrations (10% and 20%) of diet formulated with smoked fish.

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