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Modulation of biochemical stress initiated by toxicants in diet prepared with fish smoked with polyethylene (plastic) materials as fuel source

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This study investigated the oxidative modulation potentials of diets supplemented with *Solanum lycopersicum* and *Allium cepa* against biochemical changes initiated in rats fed diet prepared with fish smoked with polyethylene material (FSP) and fish smoked with firewood (FSF). Thirty male Wistar albino rats were randomly grouped into six with five rats in each group. The biochemical parameters analyzed indicated significant ($p < 0.05$) increases in the activities of liver enzymes [alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST)] and concentration of total bilirubin and malondialdehyde (MDA) in rats maintained on diets prepared with FSF and FSP only when compared to rats maintained on rats pellets only and rats maintained on the supplemented diets. These groups of rats also presented significant ($p < 0.05$) fluctuations in activities of glutathione peroxidise (GPx), superoxide dismutase (SOD) and catalase (CAT) and concentrations of total protein, albumin, total cholesterol and glutathione (GSH). However, the *S. lycopersicum* and *A. cepa* treated groups showed significant restoration towards their respective normal control values. These results indicate that smoking as a food processing method (especially using polyethylene materials) generates oxidants that may induce oxidative damage. The antioxidative role of *A. cepa and S. lycopersicum* in the diets of treated animals emphasized their hepatoprotective potentials.

Key words: Smoked fish, polyethylene, *S. lycopersicum*, *A. cepa,* oxidants, antioxidative, toxicants.

INTRODUCTION

Fish is one of the world favourite foods and a major source of dietary protein, containing essential fatty acids and essential amino acids among others. Fish can be prepared (cooked) in many different ways. Deep-fat frying, grilling, broiling, roasting, boiling, baking, smoking, stir-frying and braising are the most common methods for fish processing. A central concern of fish processing is to prevent it from deteriorating. Roasting (smoking) enhances the flavour and taste of the fish.

However, advancing scientific knowledge have shown that cooking and processing of foods at high temperatures generates genotoxic substances such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic aromatic amines (HAAs), heavy metals (Sugimura, 1997;

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Badry, 2010; Ujowundu et al., 2013, 2014a), among others. Grilling (broiling/roasting) meat, fish or other foods with intense heat over a direct flame results in fat dripping on the hot fire and yielding flames containing a number of PAHs (Agerstad and Skog, 2005; Ujowundu et al., 2014a) and HAAs. These chemicals adhere to the surface of the food, and the more intense the heat, the more PAHs and HAAs are present. The formation of these PAHs and HAAs on roasted foods is dependent on the distance of food from the heat source (Philips, 2002), fat content of the fish (Knize et al*.,* 1999), duration of roasting (Nawrot et al*.,* 1999), temperature used (World Health Organization (WHO), 1998), whether fat is allowed to drop onto the heat source and type of fuel used (SCF, 2002; Ujowundu et al., 2014b).

Our study exposed a hazardous practice by locals that involved the use of waste polyethylene materials, plastics, tyres, cartons and other inflammable materials to generate fire, heat and smoke used to process fish and meat in some parts of Nigeria, such as Owerri in Imo state (Ujowundu et al., 2014b). The use of these materials is probably to cut corners and save cost, being readily available with little or no cost, or because these materials can generate the much needed fire/flame for fast roasting.

The by-products of polyethylene or plastic combustion are airborne particulate emission (soot) and solid residue ash (black carbonaceous colour) (Graham, 2012). Several studies have demonstrated that soot and solid residue ash possess a high potential of causing significant health and environmental concern. The soot when generated is accompanied with volatile organic compounds (VOCs), semi-VOCs smoke (particulate matter), particulate bound heavy metals, PAHs, polychlorinated dibenzofurans (PCDFs) and dioxins (Valavanidid et al., 2008). These toxicants released by these materials during combustion would probably accumulate on or absorbed by the processed foods. These foods are consumed with other food materials especially those of plant origin such as fruits, spices and vegetables. The chemical and phytochemical content of these plants are important antioxidants that ameliorate the toxic effects of the contaminants/toxicants (Stanner et al*.,* 2004; Poljsak, 2011).

Solanum lycopersicum (tomato) and *Allium cepa* (onion) are such plants God has used to bless man. Several studies have indicated the positive health effects of these plants, including anticarcinogenic, antioxidative, hepatoprotective, cardioprotective, antidiabetic and antimicrobial activities, among others (Das et al*.,* 2005; Ciz et al*.,* 2008; Kobori et al*.,* 2011; Weremfo et al., 2011; Ujowundu et al., 2012, 2014b).

Consumption of tomatoes has been proposed to reduce the risk of several chronic diseases such as cardiovascular diseases and certain types of cancer, especially prostate cancer (Rao and Agarwal, 1999). Lycopene is the most prominent carotenoid in tomatoes (Karimi et al., 2005) and investigations have strengthened the hypothesis that lycopene could be a fundamental factor for the preventive effects of tomatoes and tomato products (Basu and Imrham, 2007). Although observation indicates that both tomato juice and lycopene reduced lipid peroxidation (El-Nashar and Abduljawad, 2012). Carotenoids exert antioxidants activity and lycopenes exhibits the highest overall single oxygen-quenching carotenoid, twice as that of carotene (DiMascio et al., 1989). *A. cepa* contains flavonoids and sulphuryl compounds which offers protection against cellular damage (Hodges et al., 1999). It offers direct chemoprotective roles, reduce oxidative stress and initiates production of chemical oxidative defence mechanisms by cells (Teyssier et al., 2001; Griffiths et al., 2002; Abu-El-Ezz et al., 2011). The effectiveness of *A. cepa* is adduced to the penetration of thiosulfinates and isothiocyanates in it (Block, 1985).

It is estimated that up to 2.7 million lives could potentially be saved each year if fruits and vegetables consumption are sufficiently increased (WHO, 2002). The health benefits of diet rich in vegetables have also been recognized and there are evidences that nutrient content of fruits and vegetables such as dietary fibre, folate, antioxidants, vitamins and phytochemicals are associated with low risk of cardiovascular diseases and other disorders.

Our investigation revealed that in many communities and families of developing countries, the consumption of foods roasted/smoked with hazardous materials such as polyethylene (plastic material) is common. However, the adverse health implication of this practice is scarce. This study intends to expose this and also explore the hepatoprotective activities of *S. lycopersicum* (tomato) and *A. cepa* (onion) against the adverse biochemical changes on exposure to toxic compounds present in fish smoked/roasted with polyethylene (plastic material) on male albino rats.

MATERIALS AND METHODS

Plant and fish samples

Fresh samples of *S. lycopersicum* (tomatoes) and *A. sepa* (onions) were purchased from the Relieve market, Owerri Imo State, Nigeria. These plants were identified by a plant taxonomist, Dr. F.N. Mbagwu, of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The sample were deposited at the University Herbarium with Voucher Numbers; IMSUH84 (*Allium sepa*) and IMSUH142 (*Solanum lycopersicum*). Each sample was sliced, oven-dried (at 45°C until 95% moisture was removed), homogenized to powder and stored in an air-tight container at room temperature prior to feed compounding. Fresh samples of mackerel fish (*Rastrelliger spp*) were bought from a Market at Obinze in Owerri-West. The fish samples were identified by Mr. C.F. Ezeafulukwe of the Department of Fisheries and Aquaculture, Federal University of Technology, Owerri (FUTO). The researchers adopted the methods used by the vendors to process the fish samples.

The fish samples were processed with smoke, heat and flame

Table 1. Animal groupings and feeds.

Animal grouping	Feed
PO	100% rat Pellets only
PSF	60% Pellet + 40% Fish Smoked with Firewood (FSF)
PSP	60% Pellets + 40% Fish Smoked with Polyethylene materials (FSP)
PSPS	50% Pellets + 40% FSP + 10% *S. lycopersicum*
PSPA	50% Pellets + 40% FSP + 10% *A. cepa*
PSPSA	50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa*

PO = pellets only; PSF = pellets plus fish smoked with firewood; PSP = pellets plus fish smoked with polyethylene materials; PSPS = pellets plus fish smoked with polyethylene materials plus *S. lycopersicum*; PSPA = pellets plus fish smoked with polyethylene materials plus *A. cepa*; PSPSA = pellets plus fish smoked with polyethylene materials plus *S. lycopersicum* plus *A. cepa.*

from firewood for 4 hours at high temperature. The smoked fish were then divided into two portions. One portion was roasted further with fire generated from plastic material (polyethylene), and the other with firewood only for 1 hour. Each portion of the smoked/roasted fish samples were homogenized and stored in an air-tight container at room temperature prior to feed compounding. The rat pellets (Poultry Growers Pellets) was purchased from Grand feed Nigeria Ltd. The processed *S. lycopersicum*, *A. cepa,* fish smoked with firewood, fish smoked with polyethylene and rat pellets were mixed together at varying combination and concentration to compound the rat's feed as shown in Table 1. The formulated feeds were stored in well labelled, air tight containers.

Animal groupings and treatments

Thirty (30) male Wistar Albino rats (50 to 80 g) were obtained from the animal house of the Zoology Department, University of Nigeria, Nsukka, Enugu State Nigeria. The rats were allowed free access to food (rat pellet) and water *ad libitum* during a one week acclimatization period at the Animal house of the Department of Biochemistry, Federal University of Technology, Owerri. The animals were kept in steel cages placed in a well-ventilated house conditions (photoperiod: 12 h light and dark cycle each) throughout the experimental period. This study adhered to the guidelines on the care and well-being of laboratory animals (NIH, 1985) and was approved by the ethical committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria (FUTO/BCH/EC/2013/3). The rats were randomly distributed into six groups consisting five each as presented in Table 1. The rats were maintained on these feeds for 14 days and were allowed free access to water *ad libitum.*

Processing of tissues

Twenty-four hours (day 15) after the last exposure to the formulated diets, animals were anesthetized in a dichloromethane chamber. Blood was obtained by cardiac puncture and serum obtained by blood centrifugation at 1500 × g for 10 min, at 4°C. The liver from each animal was excised, weighed and stored at 4°C prior to immediate analyses. Each liver was homogenized in potassium chloride buffer (1.15 %) with ethylenediamine tetraacetic acid (EDTA) at pH 7.4 and centrifuged for 60 min. The supernatant was used to assay oxidative stress parameters.

Determination of hepatic oxidative stress parameters

Catalase (CAT) activity was determined according to the method of Aebi (1984). Briefly, 2.5 ml of phosphate buffer, 2.0 ml of H_2O_2 and 0.5 ml of sample was added into the test tube labelled stock. To 1.0 ml portion of the reaction aliquot from stock test tube, 2 ml of dichromate acetic acid reagent was added. The absorbance of the mixture was determined at 240 nm at a minute interval into 4 places.

Superoxide dismutase (SOD) activity was determined using the method of Xin et al. (1991). Briefly, 0.9 ml of distilled water and 0.1 ml of sample was pipetted into test tubes. Afterwards, 0.1 ml of this mixture was mixed with 0.9 ml of carbonate buffer, and 75 µl of xanthine oxidase added. The absorbance was read at 500 nm for 3 min at 20 s interval. The changing rate of absorbance was used to determine the superoxide dismutase activity. Glutathione peroxidase (GPx) activity was determined by the method of Paglia and Valentine (1967). Briefly, 3.0 ml of phosphate buffer, 0.55 ml of guaiacol, 0.03 ml of H_2O_2 and 0.1 ml of sample were added into test tubes and mixed. The absorbance of the mixture was taken at 436 nm for 2 min at 30 s interval.

Glutathione concentration was determined by the method of King and Wootton (1959). Briefly, into test tubes labelled test and blank 0.1 ml of sample and 0.1 ml of distilled water were added, respectively. Also, 0.9 ml distilled water and 0.02 ml of 20% sodium sulphite were added to all test tubes, mixed and stood for 2 min at 25°C. Afterwards, 0.02 ml of lithium sulphate and 0.02 ml of 20% Na_2C0_3 were added to all test tubes and mixed. Then, 0.2 ml phosphor – 18 – tungstic acid was added to the test tubes, shook and allowed to stand further for 4 min for maximum colour development. Finally, 2.5 ml of 2% sodium sulphite was added to test tubes and the absorbance was read at 680 nm within 10 min.

The determination of malondialdehyde (MDA) concentration was by the method of Wallin et al. *(*1993). Briefly, 0.1 ml of sample, 0.9 ml of distilled water, 0.5 ml of 25% trichloroacetic acid (TCA) and 0.5 ml of 17% TBA in 0.3% NaOH were pipetted into test tubes. The mixture was incubated at 95°C for 40 min and cooled in water after incubation. Afterwards, 0.1 ml of 20% sodium dodecyl sulphate was added to the mixture. The absorbance of the mixture was determined at 532 and 600 nm against a blank.

Determination of liver function parameters

Serum alanine aminotransferase (ALT) activity was determined by Reitman and Frankel (1957) method. Briefly, 0.1 ml of serum was pipetted into test tubes labelled reagent blank and sample, and 0.5 ml of the ALT reagent I was added to test tubes labelled reagent blank and sample. Also, 0.1 ml of distilled water was added into the test tubes labelled reagent blank. All test tubes were appropriately mixed and incubated at 25°C for 20 min. Afterwards, 0.5 ml of ALT reagent II was added to all test tubes, mixed and allowed to stand

at 25°C for 20 min. Later, 5.0 ml of NaOH was added to the test tubes, mixed and absorbance read against the reagent blank after 5 min and the activity determined.

The activity of aspartate aminotransferase (AST) was determined by Reitman and Frankel (1957) and Schmidt and Schmidt (1963). Briefly, test tubes were labelled reagent blank and sample and 0.1 ml of serum was added into the test tubes labelled sample, and 0.5 ml of AST reagent I was added to all test tubes. Then, 0.1 ml of distilled water was added into the test tube labelled reagent blank. All tubes were mixed and incubated at 37°C for 30 min. Afterwards, 0.5 ml of AST reagent II (containing 2, 4 – dinitrophenylhydrazine) was added to all test tubes, appropriately mixed and allowed to stand further at 25°C 20 min. Finally, 5.0 ml of NaOH was also added to the test tubes and mixed, and the absorbance of the sample was measured against the reagent blank after 5 min.

The alkaline phosphatase (ALP) activity was determined according to the Deutsche Gesellschaft für Klinische Chemie (1972) method. Briefly, 0.01 ml of the sample was added into labelled test tubes. Then 0.50 ml of ALP reagent (containing diethanolamine buffer, magnesium chloride and p-nitrophenylphosphate) was added to the tubes and mixed. The absorbance was read against water blank at 405 nm and activity (U/l) determined.

Serum total protein (TP) concentration was determined using Tietz (1995) method. Briefly, into tubes labelled reagent blank, standard sample and sample blank were added 0.02 ml distilled water, standard protein preparation and samples. Then, 1.0 ml of total protein Reagent 1 was added to all the test tubes, except in sample blank in which Reagent 2 was added. The content of these test tubes were mixed and incubated at 25°C for 30 min. The absorbance was taken at 546 nm, and the concentration determined.

Serum albumin (ALB) concentration was determined using the method of Grant et al. (1987) and Doumas et al. (1971). Briefly, 10 µl of distilled water, standard albumin preparation and samples were added to different test tubes and 300 µl of albumin reagent containing bromocresol green (BCG) was added to the tubes mixed and incubated at 25°C for 20 min. Absorbance was read at 578 nm. The determination of the plasma globulin was by the formula:

Plasma globulin = Total protein (TP) - Plasma albumin (ALB)

Serum bilirubin was determined by the modified method of Jendrassik and Grof (1938), using Randox laboratory test Kit (Antrim, UK). Briefly, into test tubes labelled sample blank and sample, 200 µl of bilirubin reagent I was added. Afterward 50 µl of Bilirubin reagent II was added to tube labelled sample and 1000 µl of Bilirubin reagent III added to all the test tubes. Also, 200 µl of serum was added to all the test tubes. The test tubes were mixed and incubated at 25°C for 10 min. Afterwards, 1000 µl of Bilirubin reagent IV was added to all test tubes, mixed and incubated further at 25°C for 30 min. The absorbance was read against the sample blank at 578 nm, and the concentration determined. Cholesterol was determination by Allain et al. (1974) and Meiattini et al. (1978) methods for the quantitative *in vitro* determination of cholesterol concentration in plasma using biosystems kit (Barcelona, Spain). Briefly, test tubes were labelled blank, standard and sample. Then 10 ul of cholesterol standard solution and samples were pipetted into the test tubes labelled standard and sample, respectively. Afterwards 1.0 ml of cholesterol Reagent A (containing sodium cholate, phenol, cholesterol esterase, cholesterol oxidase, peroxidase and 4-amino antipyrine) was added to all test tubes. The test tubes were mixed and incubated at 25°C for 10 min and absorbance was taken at 500 nm.

Statistical analyses

The results were expressed as mean ± standard deviation, and the test of statistical significance was carried out using analysis of variance (ANOVA) at 95% confidence interval ($P \leq 0.05$). All statistical calculations were performed with SPSS 17.0 for Windows (Ozdamar, 1991).

RESULTS AND DISCUSSION

In this study the division of the smoked fish into two portions was to achieve two things; (1) to determine the effect of fish smoked with firewood only and (ii) the effect of fish smoked with firewood and polyethylene material. The use of polyethylene (plastics) is a growing trend, in local food processing in Nigeria which adverse effects needs to be highlighted.

Food can become contaminated during thermal treatments such as drying, smoking, roasting, baking frying and grilling (Ishizaki et al., 2010). Processing of food at high temperatures generates highly lipophilic compounds (PAHs) known to be potent carcinogens (Silva et al., 2011). In this study, the fishes were roasted employing traditional roasting methods with firewood and polyethylene material as the sources of fuel, except for the fresh fish samples. The levels of PAHs in smoke depends on heat sources, temperature, flame intensity, particulate materials generated during combustion, etc (Muthumbi et al., 2003; Rey-Salgueiro et al., 2004).

Our previous study showed that roasting contributed to the contamination of the fish samples with heavy metals, and this was also dependent on the source of fuel used. This is in agreement with the research of Ersoy et al. (2005) who found that cooking methods such as frying, microwaving, baking and grilling increased the concentrations of some heavy metals (Cd, Pb and Arsenic) in fish.

The present result show that the feeding of rats with smoked fish diet (40% w/w) significantly elevated ($P < 0.05$) the concentration of lipid peroxidation product (malondialdehyde) in the liver after 14 days (Figure 1). Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes (Cheeseman, 1993), by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Bergendi et al., 1999). These latter compounds then decompose to form a wide variety of products in particular malondialdehyde (MDA) (Zeyuan et al., 1998). Lipid peroxidation in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity which is essential for proper functioning of the cell (Iman, 2011). So the increase in MDA concentration (an index of lipid peroxidation) observed in the rats fed the smoked fish diets (PSF and PSP), indicates liver cell membrane damage. This is in accordance with the work of Liu et al. (2008) which recorded an increase in MDA with phenanthrene exposure. Phenanthrene is one of components observed in fish smoked with firewood, polyethylene and tyre (Ujowundu et al., 2014b). The result is also consistent with the works of Ujowundu et al. (2011), Vasanth et al.

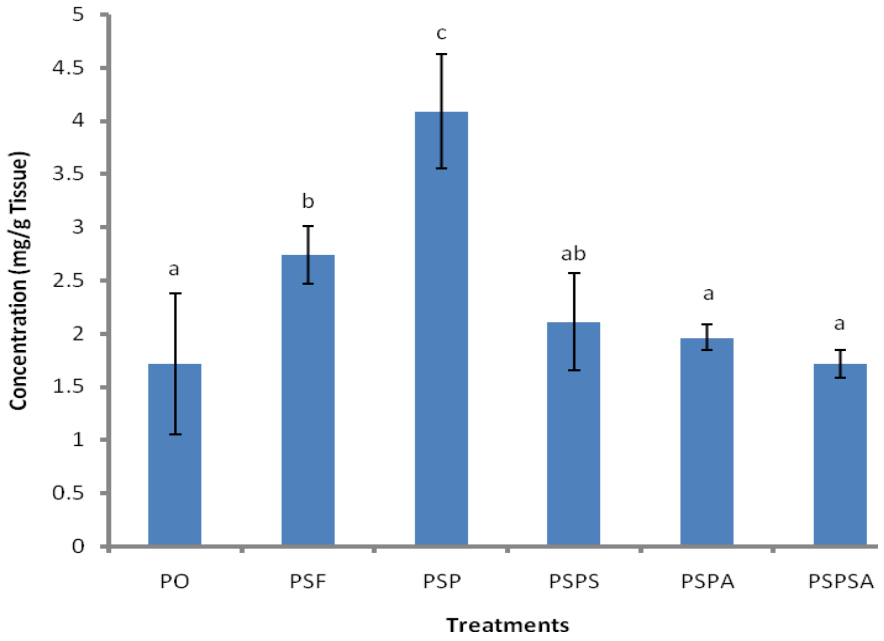

Figure 1. Concentration of MDA in liver homogenate of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

(2012) and Ujowundu et al. (2012a) who observed an increase in MDA in rats after administration of diesel petroleum, anthracene and crude petroleum oil, respectively. Treatment of the rats in this study with *A. cepa* and *S. lycopersicum* supplemented diets (PSPS, PSPA and PSPSA groups) significantly reduced the MDA concentration towards the control value, indicating suppression of oxidative stress. Greater reduction in MDA concentration was presented in the PSPSA group treated with combined supplement of *A. cepa* and *S. lycopersicum*, suggesting a synergy.

We observed a significant increase in glutathione peroxidase activity in the liver of PSP rats but a non-significant increase in PSF rats when compared to normal control (PO) rats. This increase could be a response of the organ to the oxidative stress (Iman, 2011) induced by heavy metals and the metabolites of PAHs and HAAs in the smoked fish samples. Furthermore, the increase in GPx activity (Figure 2) was accompanied by a significant reduction in glutathione (GSH) concentration (Figure 3) in the liver PSP rat group. GSH is an endogenous substance that protects cells suffering from oxidative stress (Iman, 2011). Glutathione can function as an antioxidant by catalysing the reduction of H_2O_2 to water (Abuja and Albertini, 2001). It can react with singlet oxygen, superoxide and hydroxyl radicals (Singh et al., 2003; Hashimoto et al., 2008). Glutathione also attacks eletrophilic centers and thus protects proteins, lipids and nucleic acids from the attack of electrophilic compounds which are capable of reacting with their SH groups (Hayes et al., 1991; Ahluwalia et al., 1996). The decrease in GSH concentration observed in this study corresponds to the increase in GPx activity since GSH is used in GPx pathway. The increase in GPx activity is indicative of response to the induced oxidative stress (Olagoke, 2008), due to toxic effect of toxicants, usually indicated by increase in defence enzymes (Doherty et al., 2010). Treatment with *A. cepa* and *S. lycopersicum* (groups PSPS, PSPA) resulted to non-significant decrease in the concentrations of GSH when compared to control.

The result of the present study also showed that the increase in MDA concentration in PSF and PSP was accompanied by a concomitant significant decrease in the activities SOD (Figure 4). However catalase (CAT) activity increased significantly in all the groups administered polyethylene smoked fish diets (Figure 5). SOD, CAT and GSH are crucial in the detoxification of oxy-radicals to non-reactive molecules (Van Der Oost et al., 2003). These results indicates that PAHs, heavy metals and other toxicants in the smoked fish diets, induced the production of $O_2^{\cdot-}$ and H_2O_2 which are substrates of SOD and CAT (Vasanth et al., 2012). The results of this study indicate that in rats fed the

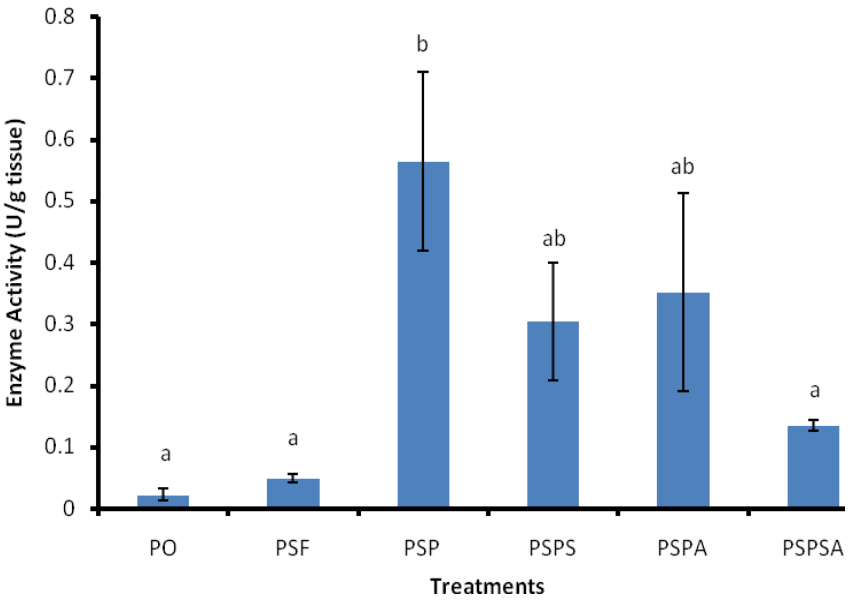

Figure 2. Activities of GPx in liver homogenate of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

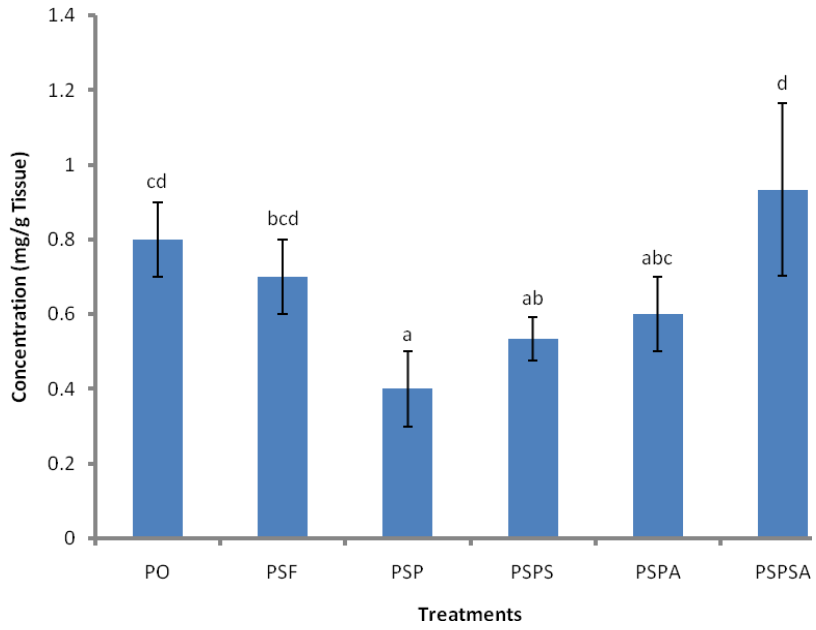

Figure 3. Concentration of GSH in liver homogenate of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

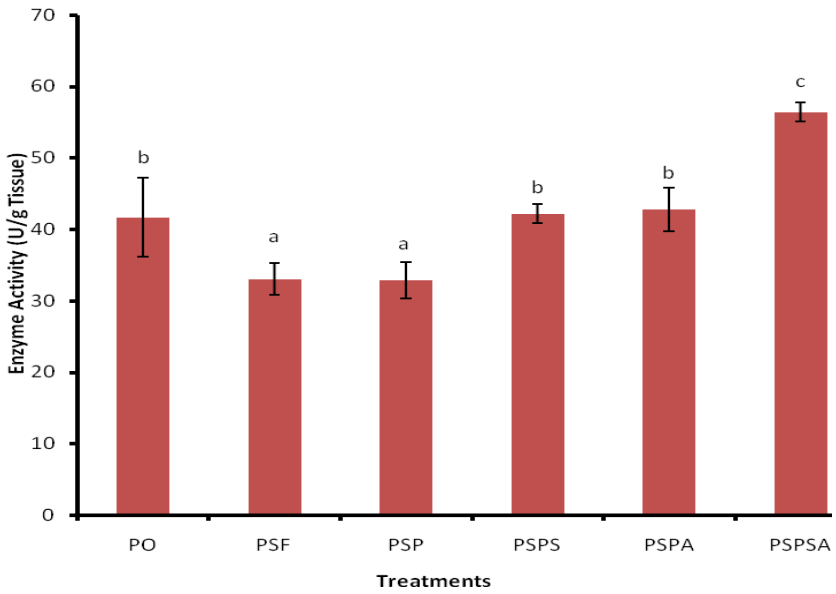

Figure 4. Activities of SOD in liver homogenate of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% Pellet + 40% fish smoked with firewood; PSP = 60% Pellets + 40% fish smoked with polyethylene materials; PSPS = 50% Pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

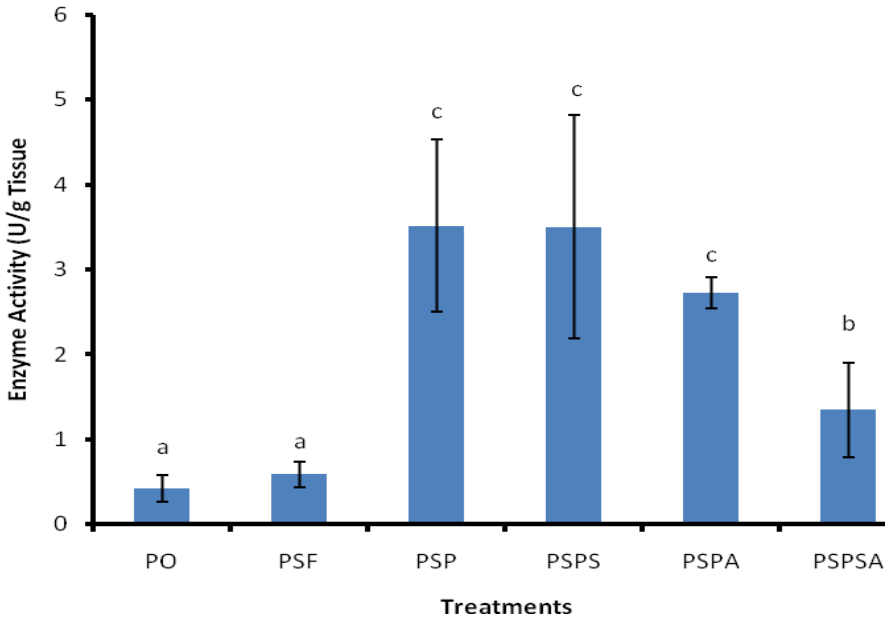

Figure 5. Activities of catalase in liver homogenate of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa.* Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only, PSF = 60% pellet + 40% fish smoked with firewood, PSP = 60% pellets + 40% fish smoked with polyethylene materials, PSPS = 50% Pellets + 40% FSP + 10% *S. lycopersicum,* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*, PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

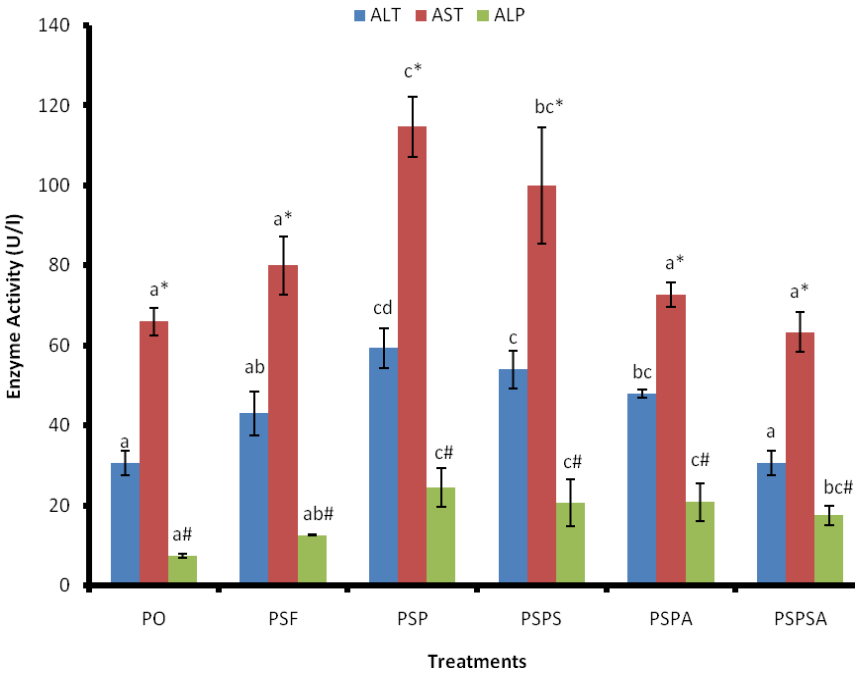

Figure 6. Activities of ALT, AST and ALP in serum of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only, PSF = 60% Pellet + 40% fish smoked with firewood, PSP = 60% pellets + 40% fish smoked with polyethylene materials, PSPS = 50% Pellets + 40% FSP + 10% *S. lycopersicum,* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*, PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

polyethylene smoked fish diets, SOD, CAT and GPx were induced. It therefore implies that a considerable amount of $O_2^{\cdot-}$ was produced originating abundant H_2O_2 that needs to be detoxified by both CAT and GPx pathways. This is in agreement with the work of Vasanth et al. (2012) who found that SOD and CAT were induced following administration of anthracene to laboratory animals. Then, since oxidative stress due to the toxicants is usually indicated by increased levels of products of oxidative damage (MDA) and subsequent increase in defence enzymes (GPx, SOD and CAT) in response to the stress (Doherty et al., 2010) or decrease due to overwhelming effect of the pollutants (Faramobi et al., 2007; Olagoke 2008; Ujowundu et al., 2011), it is then sufficient to conclude that the decrease in the activity of SOD is due to the overwhelming effect of the toxicants from the smoked fish diets where the system used the SOD to detoxify the resulting superoxide radicals.

The activities of these enzymes (CAT, SOD and GPx) were restored towards normal in the *A. cepa* and *S. lycopersicum* treated groups (PSPS, PSPA and PSPSA), suggesting the protective effect of *A. cepa* and *S. lycopersicum*. The active constituents of the plants might have caused a stabilization and repair of plasma membranes damaged by exposure to the toxicants in the roasted samples (Thabrew et al., 1987). However, the combined treatment with *A. cepa* and *S. lycopersicum* was more effective in restoring the antioxidant enzymes to normal, probably due the phenomenon/effect of synergy when compared to the individual actions of *A. cepa* and *S. lycopersicum.*

Accumulation of PAH compounds in the liver, kidney and other organs might have caused serious pathological damage due to the exposure (Vutukuru et al., 2007). When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the bloodstream making the enzymes activities in the blood to increase. Measurement of the activities of serum (plasma) marker enzymes like AST, ALT, and ALP as well as levels of serum (plasma) total bilirubin have proved a powerful tool for the assessment of liver function (Ulican et al., 2003; Porchezhian and Ansari, 2005). The significant increase in the plasma activities of ALT, AST, ALP (Figure 6) and concentration of total bilirubin (TBil) (Figure 7) observed in the rats fed with PSP is indicative of liver damage induced by the toxicants (PAHs and Heavy metals) in the smoked fish diet. The significant increase in these enzymes could either be due to their possible leakage from the cytosol across damaged plasma membrane into the general

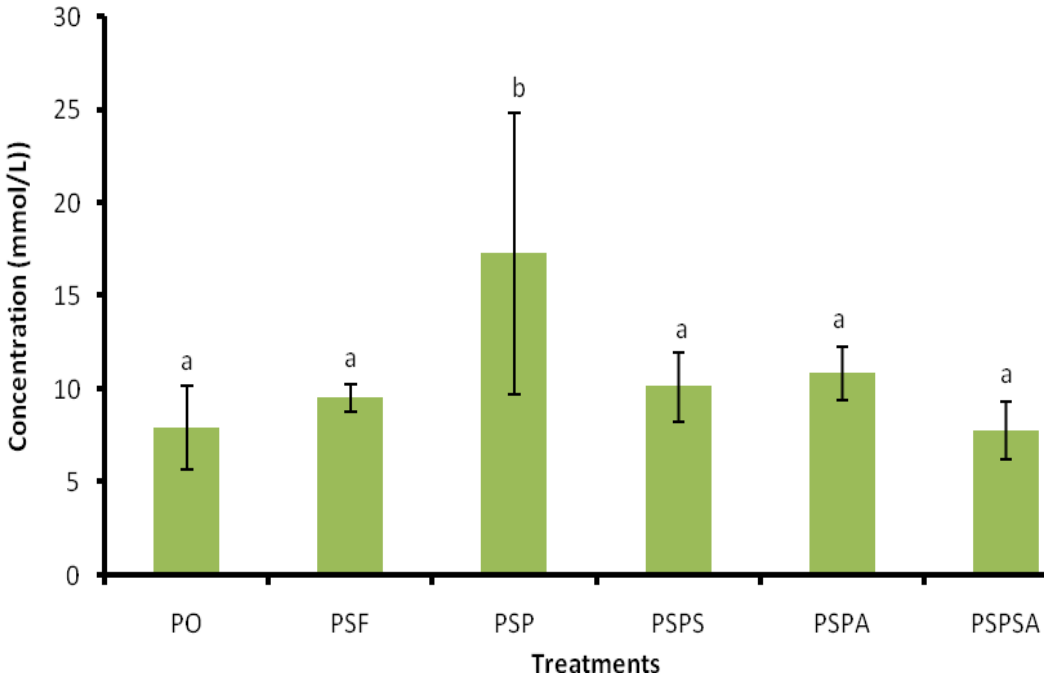

Figure 7. Serum Bilirubin concentration in rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

blood circulation or increase in their synthesis as a result of the organ dysfunction (Ploa and Hewitt, 1989; Vasanth et al., 2012). It could also be due to induction of necrotic lesions in the hepatocytes of the rats (Ujowundu et al., 2011). Thus the elevated enzymes activities could be considered to be manifestation of oxidative stress (Vasanth et al., 2012) caused by the toxicants in the smoked fish diets (Ujowundu et al., 2014). These results demonstrated that both *A. cepa* (onion) and *S. lycopersicum* (tomato) exhibited hepatoprotective action against toxicants in the smoked fish diet. The reduced activities of ALT and AST and concentration of total bilirubin in groups which diets were supplemented with *A. cepa* and *S. lycopersicum* toward the normal control values indicates stabilization of plasma membrane and/or repair of damaged hepatic tissues. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987). The plants mediated suppression of the increased ALP activity with the concurrent reduction of raised bilirubin level which suggests the possibility of the plants constituents being able to stabilize biliary dysfunction in the rat liver in sub-acute hepatic injury by the toxicants. This agrees with the work of Weremfo et al. (2011) which investigated the hepatoprotective activity of tomato pulp against carbon tetrachloride-induced hepatic damage in rats. However, *A. cepa* significantly ($P < 0.05$) showed much efficacy than *S. lycopersicum* in restoring AST and ALT activities to normal, while *S. lycopersicum* was slightly more effective in reducing the levels of total bilirubin and ALP to normal. Nevertheless, the combined *A. cepa* and *S. lycopersicum* action dominated in effectiveness than the individual plants.

More so, there was significant decrease ($P < 0.05$) in albumin (Alb) and total protein (TP) (Figure 8) in the PSF and PSP when compared to the control (PO). Albumin specifically synthesized by the liver, is among the major antioxidant components of the plasma, and might play a major role of the total antioxidant capacity of plasma (Aycicek et al., 2005; Ujowundu et al., 2012b). The total protein, albumin and globulin level may decrease due to liver dysfunction, malnutrition and malabsorption, diarrhoea, nephrosis and acute haemolytic anaemia (Ekam et al., 2012). The decrease in the concentration of albumin and total protein may be due to compromised synthetic function of the liver induced by oxidative stress. Another factor that may be responsible for the reduction albumin, is its role in binding a host of endogenous and exogenous substances (xenobiotics) while acting as an antioxidant. This important molecules might have been used to scavenge the resulting free radicals and heavy

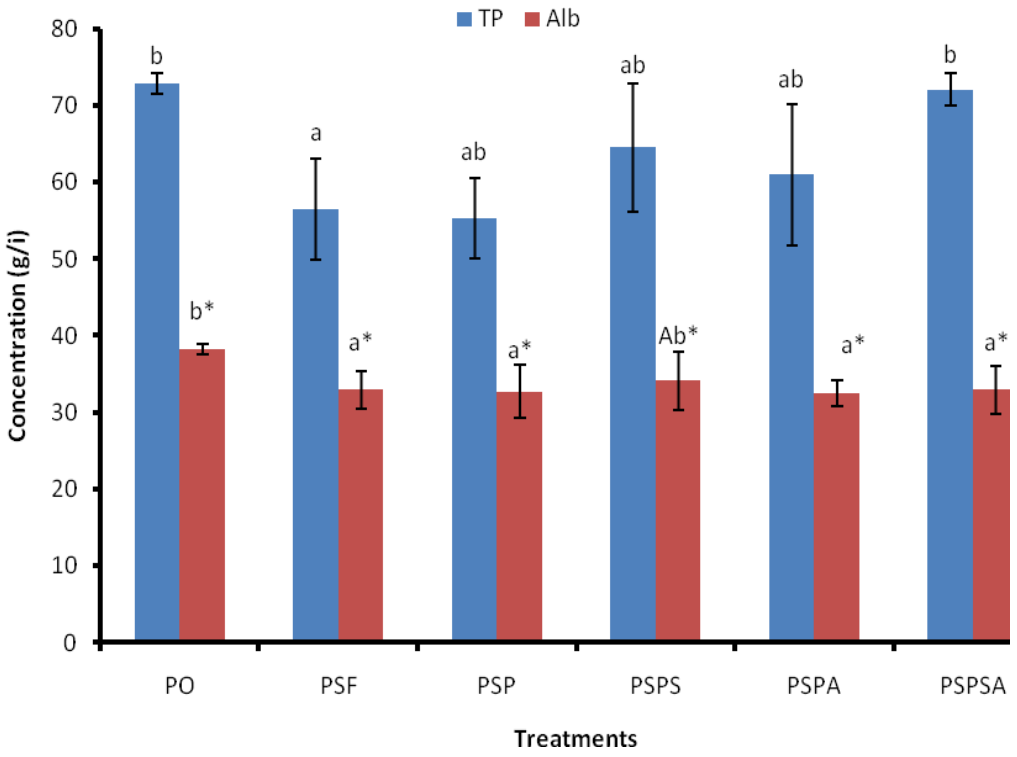

Figure 8. Concentrations of Total Protein (TP) and Albumin (Alb) in serum of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

metals (toxicants from the roasted fish). The toxic ROS load might have exceeded the capacity of the animals' antioxidant systems, causing albumin to be used, leading to its reduction. The decrease in the concentrations of albumin and total protein in the study is corroborated with the work of Nwaogu and Onyeze (2010) and Ujowundu et al. (2012b). Higher concentrations of these proteins were observed in plants-treated groups (PSPS, PSPA and PSPSA), suggesting a hepato-protection by *A. cepa* and *S. lycopersicum*. *S. lycopersicum* showed better ability than *A. cepa* in restoring these proteins to normal values. However the combined *A. cepa* and *S. lycopersicum* supplemented diet proved to be much more effective than the individual treatment. This suggests that the constituents of the plants (*A. cepa* and *S. lycopersicum*) could have worked synergistically.

Furthermore, the result of the study shows significant decrease ($P < 0.05$) in the cholesterol concentration in the PSF and PSP fed rats (Figure 9) when compared with the control. Cholesterol is primarily synthesized by the liver. Hence, the reduction in the concentration of cholesterol in the intoxicated groups may indicate a decrease in the synthetic function of the liver caused by oxidative stress (Poulos et al., 1973). Diet supplement with *A. cepa* and *S. lycopersicum* did not show much restoration potential in the rats when compared to the cholesterol concentration in the intoxicated rats.

Conclusion

From our findings, it can be concluded that roasting of fish, especially using polyethylene materials, generates a lot of toxicants. However, phytochemicals derived from plant such as *A. cepa* and *S. lycopersicum* can act as antioxidants. Because they act as free radical scavengers, singlet oxygen quenchers or metal chelators, consumption of plant products possessing antioxidant potential protects humans from the oxidative damage of reactive oxygen species (ROS).

Conflict of Interests

The authors have not declared any conflict of interests.

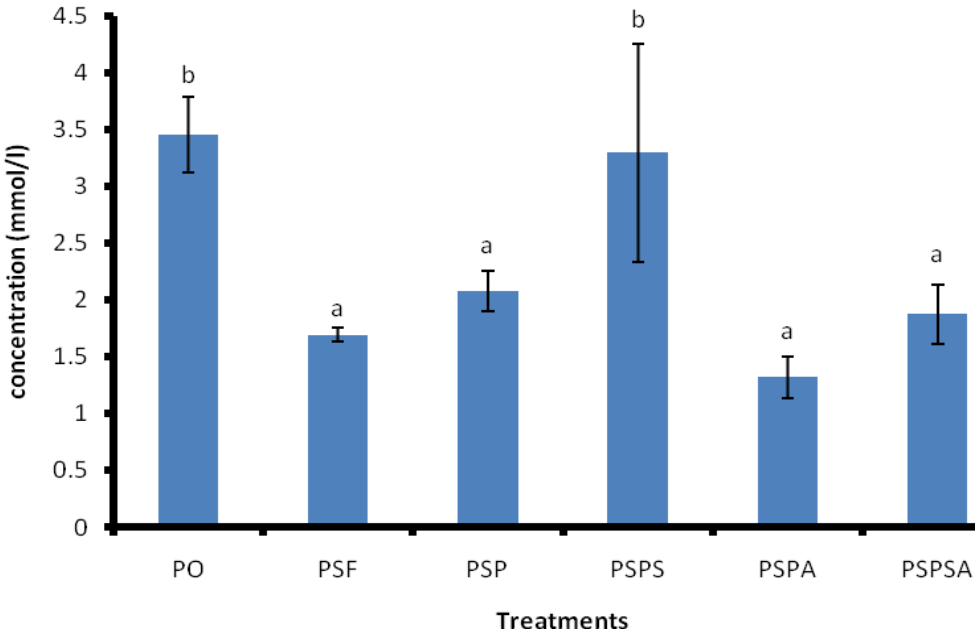

Figure 9. Serum Cholesterol of rats maintained on diet prepared with fish smoked with polyethylene (plastics) and the antioxidative role of *S. lycopersicum* and *A. cepa*. Each bar represents the mean ± SD. Bars with different letters are significantly different ($p < 0.05$). PO = Pellets only; PSF = 60% pellet + 40% fish smoked with firewood; PSP = 60% pellets + 40% Fish smoked with polyethylene materials; PSPS = 50% pellets + 40% FSP + 10% *S. lycopersicum;* PSPA = 50% pellets + 40% FSP + 10% *A. cepa*; PSPSA = 50% pellets + 40% FSP + 5% *S. lycopersicum* + 5% *A. cepa.*

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