

Original Research Paper

Chemoprotective Effects of Crude Extracts of Lycopene and Flavonoid in Rats Exposed to Locally Roasted Beef

ABSTRACT

This study was carried out to examine the hepatoprotective effect of crude lycopene and flavonoid extracted from tomato and onion respectively, on the biochemical changes induced by feed formulated with 15% roasted beef and 85% rats pellets. Doses of 500 mg/kg body weight of both lycopene and flavonoid were administered orally. The hepatic activities of aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) and concentrations of albumin, total protein, globulin were monitored in the animals. Also the effects of the feeding and treatment on oxidative stress parameters (CAT, SOD, GPx, Glutathion, and MDA) were determined. The changes observed were discussed.

Key words; PAHs, Heterocyclic aromatic amines, beef, toxicants, oxidative stress, live damage

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HCA) are secondary carcinogens which can be bioactivated to carcinogens in the body by cytochrome P₄₅₀ isoforms CYP1A/A2 and CYP2E1 respectively (Felton *et al.*, 2002). Studies have shown that heat processed meats (beef) at high temperatures can generate these genotoxic substances or toxicants (PAH and HCA) (Truswell *et al.*, 2002, Ujowundu *et al.* 2013). PAHs can also enter the environment through natural sources such as oil seeps and forest fires and through a variety of anthropogenic activities. These include the burning of fossil fuels and wood, smelting of metals, petroleum refining, gas flaring and petroleum spills (Douben, 2003; Latimer and Zheng; 2003, Ujowundu *et al.*...2013)

Roasting or grilling 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 and HCAs. These chemicals are formed when meat is cooked at very high temperature (100⁰C - 200⁰C) and at longer duration can greatly increase the PAH and HCA concentration especially in fatty and protein foods respectively (Larsson, 1986; Adamson, 1990; Felton and Knize, 1990; Skog, 1993). HCA generating reactions are favoured in meat or protein containing residue. Here, creatine is converted to creatinine which undergoes reaction with amino acids like phenylalanine, threonine, or alanine to form HCAs, sugars may increase the reaction rate or change the end product, but are not essential for HCA formation.

Benzo (a) pyrene (B(a)P) is recognized as a marker of PAH contamination (IARC,1993; Panalaks, 1996; Agerstad *et al.*, 2005). Benzo(a)pyrene toxicity occurs by the indirect attack on DNA directly, 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 like proteins, lipids and DNA (Dipple *et al.*, 1984).

Plants are known to contain many different components that act as antioxidants; scavenging free radicals, quenching singlet oxygen and chelating metals. The consumption of plant products possessing antioxidant potential may protect organisms from oxidative damage by reactive oxygen species (Roy *et al.*, 2007).

As a result of the nutritional role of beef in the average Nigerian diet and the methods of its processing, we examined the effect of compounds generated in heat/flame /smoke processed beef and the protective effects of crude extracts of lycopene from tomato and flavonoid from onion.

2. MATERIAL AND METHODS

Procurement / Preparation of Tomatoes, Onions and Beef

Fresh tomatoes and onion bulbs were bought from Ekeonuwa market Owerri West Local Government Area Imo State. These were washed and stored until used. The meat was purchased at a market in Obinze Owerri West Local Government Area. The meat consists of fat and fibrous parts which were processed by exposing to direct flame and smoke generated by firewood for 4 consecutive days to induce PAHs. The fresh tomatoes and onions were washed with distilled water, homogenized and oven dried at reduced temperature (40 °C) to eliminate moisture. About 200 g of the dried tomatoes and onions were subjected to Soxhlet extraction with 180 ml of ethylacetate (for lycopene extraction) and methanol (for flavonoid extraction) for tomato and onion respectively. A jel-like residue was obtained after the elimination of the solvents.

Experimental Design

Thirty (30) male Wistar albino rats were divided into five groups of six rats each. The rats were housed in steel cages and allowed to acclimatize for two weeks. The feed was formulated by mixing 15 % roasted ground beef sample and 85 % rats pellets. The treated rats were administered orally, 500 mg/kg body weight (bw) of tomato and onion extracts respectively. Also, a combined dose of 500 mg/kg bw of tomato and onion extract (250 mg each) was also used to assess synergy or dysnergy. The extracts were given with the aid of an intubator for 21 consecutive days. Feeds and water were allowed *ad libitum*. The experimental setup was thus;

Group I (negative control), were fed normal rat pellets only.

Group II (positive control), were fed formulated diet only.

Group III were fed formulated diet and onion extract in vehicle (olive oil).

Group IV were fed formulated diet and tomato extract in vehicle.

Group V were fed formulated diet and tomato + onion extract.

Biochemical Studies

The animals were scarified at the 22nd day after 24 hours fast. Blood was collected by cardiac puncture with needle and 10 ml syringe. The blood was transferred into anticoagulant free bottle. Afterwards, the blood was centrifuged to separate serum, which was used for different biochemical tests. The method of Reitman and Frankel (1957) was used to determine serum aspartate amino-transferase (AST) and alanine amino transferase (ALT) activities. Serum alkaline phosphate (ALP) was determined by the method of Englehardt *et al.* (1970). The method of Tietz (1995) was used to determine total protein and

the method of Doumas *et al*, (1971) was used to determine the albumin concentration. Catalase was estimated by the method of Aebi (1983). Superoxide Dismutase (SOD) was determined using the method of Xin *et al* (1991). Glutathione concentration was determined according to King and Wootton (1959). Glutathione peroxidase (GPx) activity was estimated as described by Paglia and Valentine (1967). Lipid peroxidation was determined spectrophotometrically by measuring the concentration of Malondialdehyde (MDA) by the method of Wallin *et al.*, (1993).

3. RESULTS

The effect of polycyclic aromatic hydrocarbon (PAH) in charred meat and the protective effect of both lycopene in tomato and flavonoid in onion on liver function markers are shown below.

Figure 1 shows treatment dependent decrease in ALT activity; ALT activity of 16.33 ± 3.79 IU/L was observed in group III, 14.00 ± 1.00 IU/L in group IV and 15.00 ± 1.00 IU/L in group V compared to group II (fed formulated diet without treatment) with ALT activity 25.00 ± 3.67 IU/L. In group I, ALT activity of 11.33 ± 3.00 IU/L is less than that in groups III, IV and V which were feed the formulated diet and treatment with the extracts. AST activity (Figure 1) ranged from 60.87 ± 12.22 IU/L in group IV to 73.3 ± 14.75 IU/L in group V, compared to 114.00 ± 11.14 IU/L in group II. The 66.67 ± 8.33 IU/L observed in group I is within the range observed in the treated groups (III, IV and V). Figure 2 showed a treatment dependant decrease in activities of ALP. With activity of 330.67 ± 26.63 IU/L in group III; 311.67 ± 10.50 IU/L in group IV and 233.33 ± 37.87 IU/L in group V, when compared with the 500.00 ± 25.94 IU/L in group II. Also, ALP activity of group I rats were lower, when compared with groups II, III, IV and V respectively.

Figure 3 also shows that the protein concentration of group II rats were decreased compared to the rats in groups I, III-V which shows non-significant change on serum protein concentration. Table 1 shows that catalase, superoxide dismutase and glutathione peroxidase activities and concentration of glutathione of positive control (group II) rats were significantly reduced when compared to rats in the treated groups. However the concentration of malondialdehyde was significantly increased in group II compared to other groups.

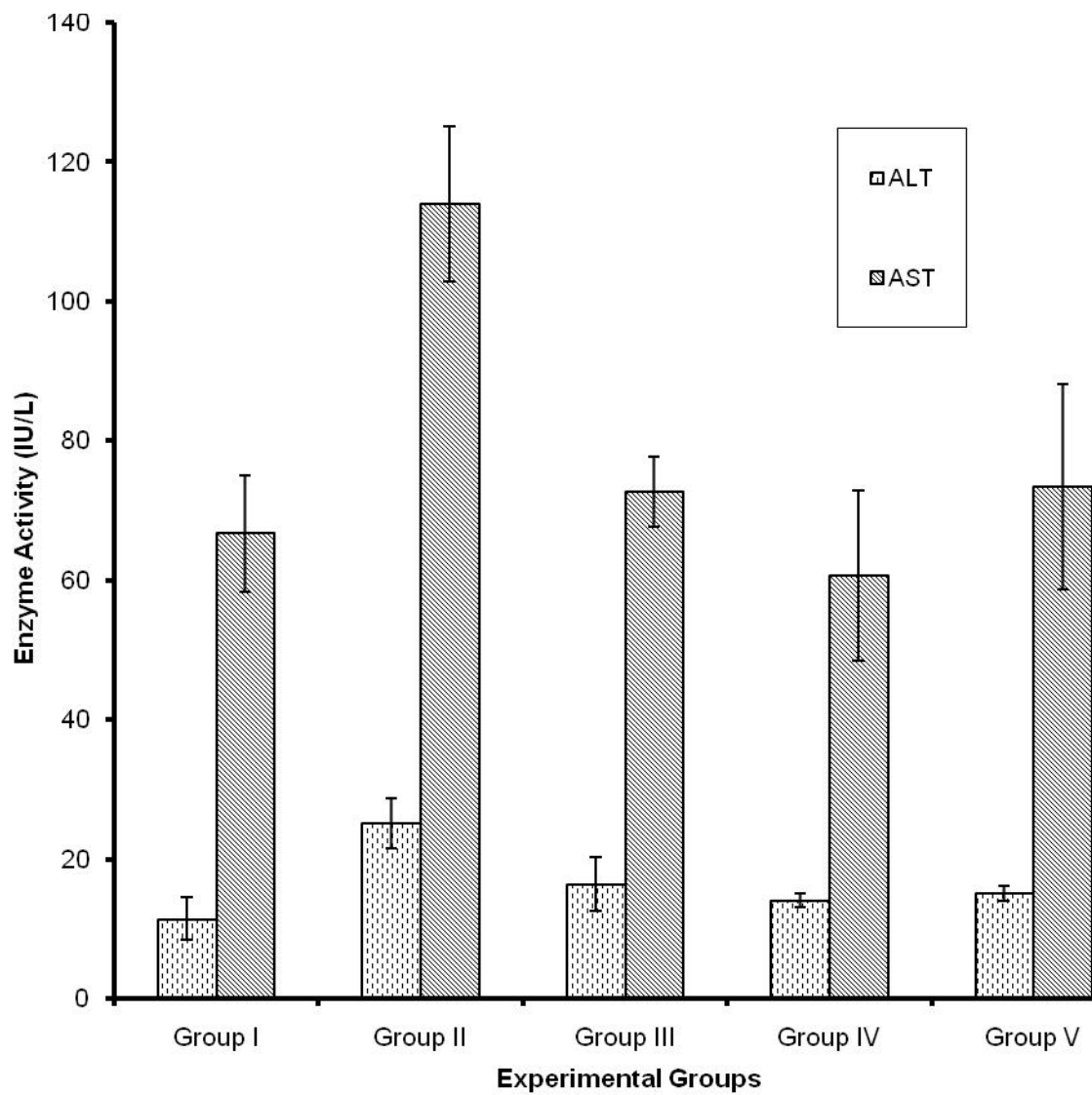


Figure 1: Enzyme Activities of Alanine Transaminase and Aspartate Transaminase

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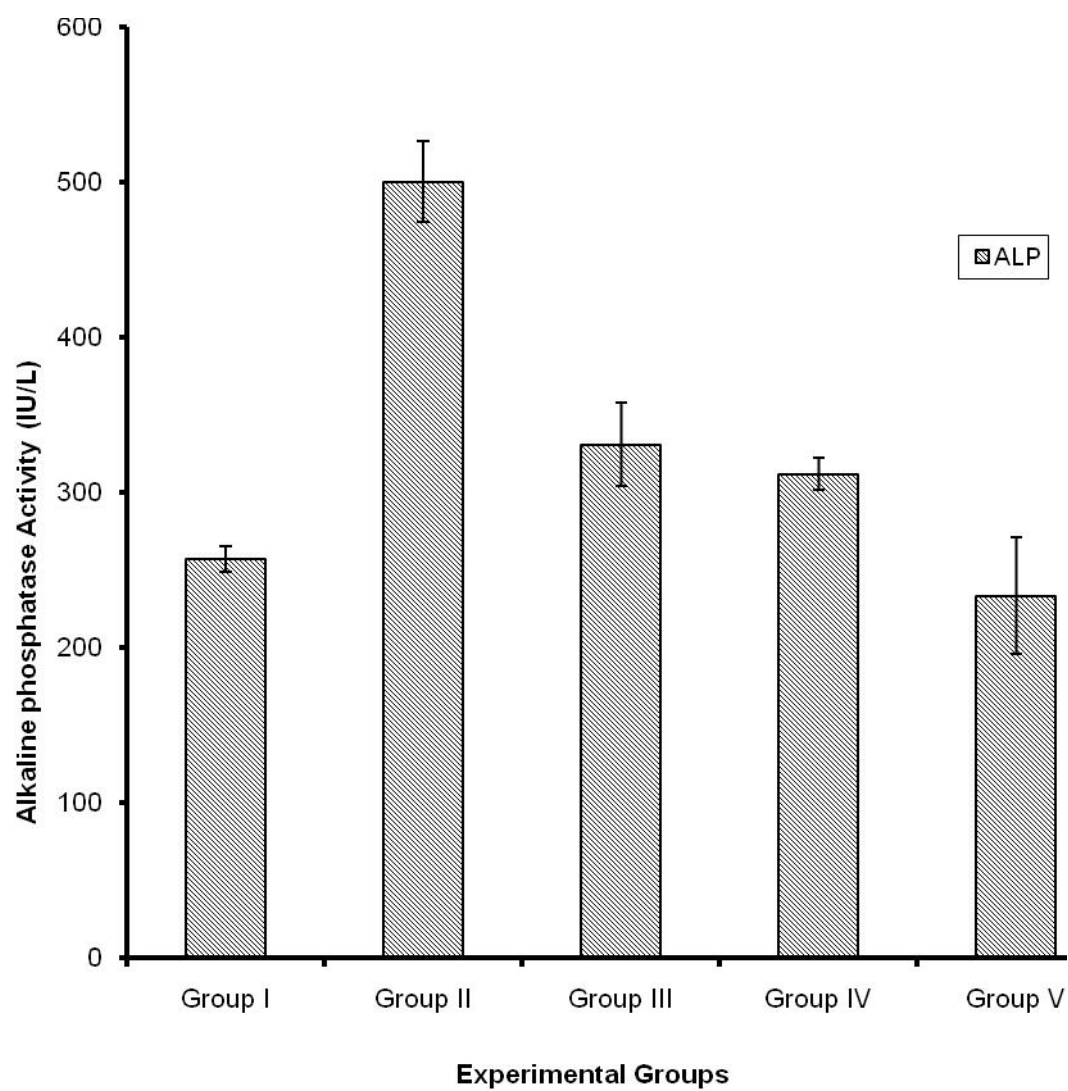


Figure 2: Enzyme activity of Alkaline Phosphatase

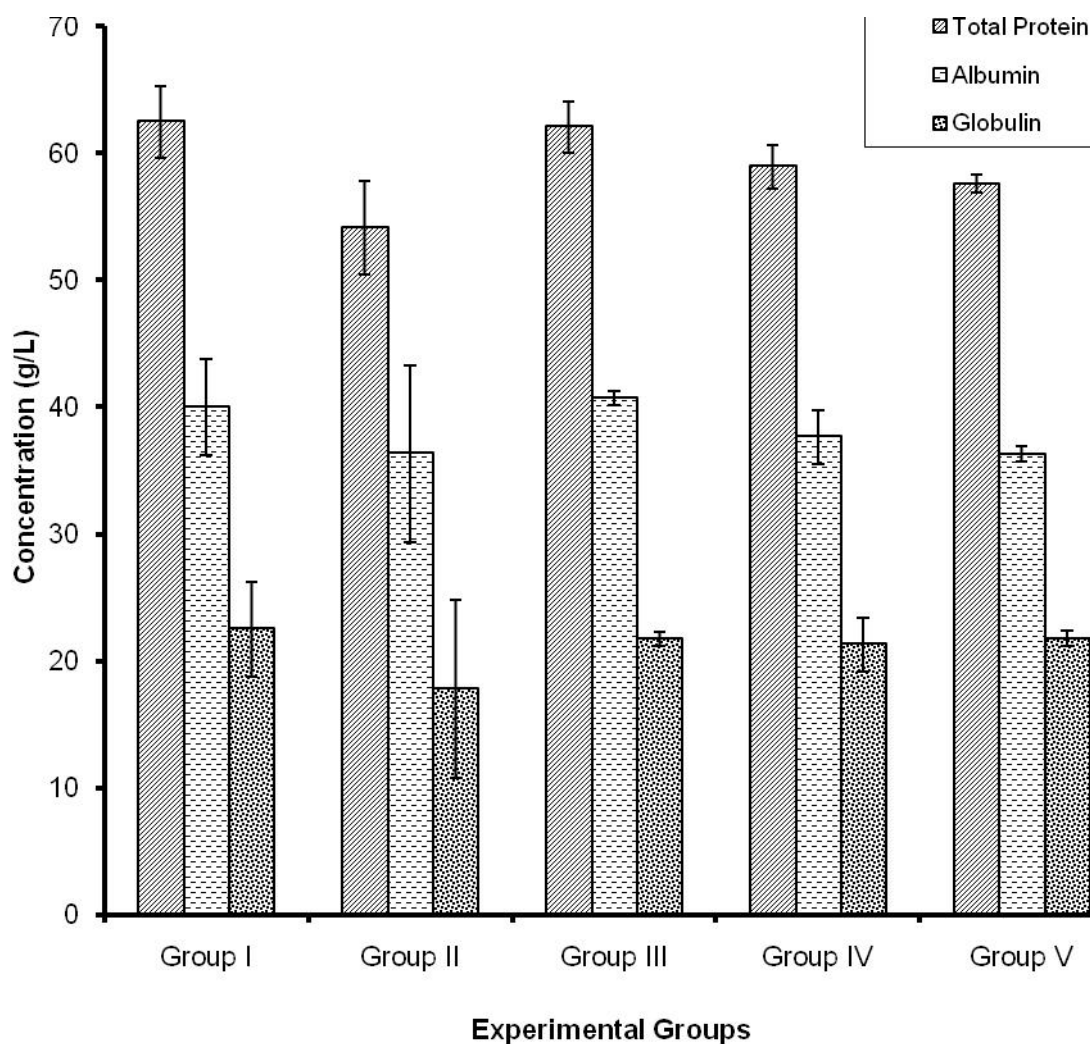


Figure 3: Serum Protein Concentration of Experimental Rats

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123 Table 1: Effect of treatment on oxidative Stress Parameters

GROUPS	CAT	SOD	GPX	GSH	MDA
Group I	6.03±0.02 ^b	8.58±.46 ^a	352.89±6.37 ^a	97.67±1.53 ^a	50.33±2.52 ^a
Group II	5.29±0.43 ^a	3.72±.015 ^b	201.56±3.79 ^b	72.33±5.13 ^d	58.33±1.53 ^b
Group III	6.07±0.035 ^b	7.62±1.83 ^a	248.27±8.49 ^{ct}	76.67±5.86 ^d	33.33±4.16 ^{ct}
Group IV	6.55±0.86 ^b	8.07±1.05 ^a	298.89±10.07 ^d	90.00±1.00 ^b	40.67±7.02 ^d

Group V	6.11±0.036 ^b	8.01±0.52 ^a	233.70±9.86 ^{ef}	91.67±1.53 ^{ab}	28.33±1.53 ^{ef}
F-Value	3.37	11.78	162.633	25.867	28.955
P-Value	0.054	0.001	0.000	0.000	0.000

These values are mean ± SD of triplicate determinations. Values in each column with different superscripts are significantly (P<0.05) different.

4. Discussion

Oxidative stress results to a biological system being forced into a highly activated state due to a loss of control of its regulatory abilities.

Prolonged oxidative stress may lead to oxidative damage of cellular DNA, proteins, lipoproteins and lipids (Pool-Zobel *et al.*, 1997; Agarwal and Rao, 1998)

Some toxic substances such PAH, HCA, heavy metals and other biochemically important constituents are generated during heat processing of beef using firewood (Truswell *et al.*, 2002; Ujowundu *et al.*, 2013). These compounds can lead to oxidative liver damage (Hard, *et al.*, 1993). The values obtained for liver function enzymes and compounds (ALT, AST and ALP) in the positive control (PC) rats suggest increased enzyme activities and this is indicative of cellular leakage and liver membrane dysfunction (Mukherjee, 2003).

The reactive metabolites (epoxides) formed as result of beef processing are highly reactive and participate in the generation of hydroxyl radicals which are the most reactive oxygen species (ROS). The radical cationic form of B(a)P, a PAH generated in heat processed beef (Ujowundu *et al.*, 2013), usually are metabolic activator of DNA adducts formation of (Dipple *et al.*, 1984). B(a)P disturbs the antioxidant defense system and can cause adverse effects due to redox cycling with their semiquinone radical, increasing oxidative stress and DNA damage (Valko *et al.* 2007). The biochemical impact of these chemical constituents can be appreciated by the values obtained for oxidative stress parameters determined. The activities of catalase (Cat), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly (P<0.05) reduced in rats fed the formulated (processed beef + rat pellets) diet only. These reductions in antioxidant enzymes activities can be attributed to the free radical scavenging role at the site of liver injury leading to their exhaustion. The contents (PAH, HCA, heavy metals etc.) in the formulated diet react readily with most hepatocellular components inducing hepatotoxicity due to epoxide formation that alkylates cellular protein and other macromolecules (Ostrowska, *et al.*, 2004). These metabolites are free radicals which could simultaneous attack polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage (Sanmugapriya, *et al.*, 2006). Lipids are extremely susceptible ROS, due to the peroxidation polyunsaturated fatty acids abundant in cells (Halliwell *et al.*, 1993). The significantly increased (P<0.05) value of lipid peroxidation product-MDA observed in the rats fed the formulated diet only, corroborates these assertions. The shortfall in the activities of important enzymes involved in radical scavenging cascade could result to elevated free radical load and oxidative stress on tissues and organs. This biochemical stress could damage the structural integrity of liver cells (Rosen and Keefa, 2000; Friedman *et al.*, 2003; Chenoweth and Hake, 1962), leading to elevated liver enzymes in the blood (Ashok *et al.*, 2002) as seen the results obtained. Under condition of severe oxidative stress, free radical generation may lead to protein modification and damage (Narasimhanaidu, *et al.*, 2006). This may be attributed to observed significant decrease (P<0.05) in total protein in the PC rats which indirectly affected the concentration of albumin and globulin.

The protective effect of the crude extracts of lycopene and flavonoids on liver cells is indicated by the reduction in the activities of these enzymes in the extracellular milieu of the liver cells in the treated rats which significantly decreased (P<0.05) or came close to the

activities of similar enzymes in the negative control (NC). The crude extracts of lycopene and flavonoid may have contributed in restoration of the liver cell membrane permeability (Kalab and Krechler, 1997). Lycopene and/or flavonoid exert its effect through antioxidant mechanism (Van et al., 1995; Agarwal and Rao, 2000; Heber and Lu, 2002; Rao et al., 2006), immune system modulation, carcinogen metabolism and metabolic pathways involving phase II drug-metabolizing enzymes (Wertz *et al.*, 2004). Quercetin is a very active flavonoid extracted from onion and can protect cells against ROS induced damage (Grace, 1994). Quercetin can scavenge free radicals through the inhibition of LDL oxidation (Fraga *et al.*, 1987; Kerry *et al.*, 1997), protecting the organism against atherosclerosis. The significantly increased ($P<0.05$) activities of CAT, SOD and GPx observed in the crude extracts treated rats suggest amelioration in the effects of the toxic content and toxic metabolites resulting from the reactions of beef content and cellular macromolecules. Metabolically, lycopene is shown to be twice as effective as β -carotene and ten times more effective than α -tocopherol in quenching reactive oxygen species, especially singlet oxygen (Stahl and Sies, 1993; Agarwal and Rao, 2000). The all-trans conjugated and non-conjugated linear structure is also important in its antioxidant actions. Quercetins can interfere with inducible nitric oxide synthase activity, resulting in reduction in nitric oxide (NO) production (Shoskes, 1998). Quercetin ability to scavenge available free radicals reduces the tendency for its reaction with nitric oxide, resulting in less damage (Shukento *et al.*, 1990; Van et al., 1995). When nitric oxide reacts with free radicals, the highly toxic peroxynitrite produced, can directly oxidize LDLs resulting in irreversible damage to cell membranes.

The effects of the extracts are also shown by the significantly reduced ($p<0.05$) concentration of MDA, a lipid peroxidation product and the increased concentration of glutathione in the extracts treated rats. Lycopene is assumed to scavenge peroxy radicals by its addition to the long polyene chain (Woodall *et al.*, 1997). This is in agreement with the findings of Kataria and Singh, (1997) and Mathur, (1994) who reported that herbs have hepatoprotective effect on chemically induced hepatic damage in rats. Quercetin seems to inhibit xanthine oxidase activity thereby resulting in decreased oxidative injury.

The crude extracts of flavonoid and/or lycopene showed some kind of ameliorative effect on the rats by the elevation of the serum total protein, albumin and globulin concentrations. This is in agreement with the findings of Faiyaz and Asna, (2010) who discovered that *Freeus racemoza* possesses potent hepatoprotective effects against carbon tetrachloride (CCl_4) induced hepatic damage in rats.

4.2 CONCLUSION

This study has shown that rats fed meats processed by Nigerians' traditional meat preparation (commonly called 'suya') induces reactive metabolites which lead to hepatic damage and other debilitating consequences. Treatment with antioxidants constituents in tomato and onion ameliorated the damage by possibly scavenging the reactive metabolites induced by PAH and other toxicants present in processed meat, reducing liver dysfunction. We recommend that to prevent oxidative stress related damage in the liver, the most pivotal action would seem to be to minimize exposure to endogenous and exogenous sources of oxidative stress by regulating and improving the traditional method of food preparation and preservation which include smoking and frying which are possible ways of incorporating PAH and other potent toxicants into the food.

222 **COMPETING INTERESTS**

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224 Authors have declared that no competing interests exist..

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