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Hepatoprotective Effects of Crude Extracts of Tomato and Onion in Rats Exposed to Locally Processed Beef

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Authors' contributions

This work was carried out in collaboration between all authors. This study was made possible by the contributions of these authors. Authors COU, HNO and FNU designed the study, performed the statistical analysis and wrote the protocol. Author HNO wrote the first draft of the manuscript. Authors COU, HNO, DCB, FNU and KOI managed the analyses of the study. Authors COU, HNO and FNU managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study was carried out to examine the hepatoprotective effect of ethylacetate extract of tomatoes and methanol extract of onions 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 extracts were administered orally. The hepatic activities of aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP) were examined, also concentrations of total protein, albumin and globulin were monitored in the animals. The effects of feeding and treatment on oxidative stress parameters (CAT, SOD, GPx, Glutathione and MDA) were determined. The changes observed were discussed.

Keywords: PAHs; Heterocyclic aromatic amines; beef; toxicants; oxidative stress; live damage.

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1. INTRODUCTION

Polycyclic aromatic hydrocarbon (PAH) and heterocyclic amine (HCA) 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 meats (beef) at high temperatures can generate these genotoxic substances or toxicants (PAH and HCA) [2,3]. 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 [4-6].

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 (100ºC - 200ºC) and at longer duration can greatly increase the PAH and HCA concentration especially in fatty and protein foods respectively [7-10]. The syntheses of heterocyclic amines (HCAs) are favoured in meat or protein containing food materials. Here, creatine is converted to creatinine which undergoes reaction with amino acids like phenylalanine, threonine or alanine to form HCAs. The presence of mono- and disaccharides 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 maker of PAH contamination [11-13]. 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 [14].

Plants are known to contain many different components that act as antioxidants; scavenging free radicals, quenching singlet oxygen and chelating metals. Studies have shown that dietary supplement of fruits and vegetables have been linked to a rise in plasma antioxidant level [15].The consumption of plant products possessing antioxidant potential may protect organisms from oxidative damage by reactive oxygen species [16]. Onions (*Allium cepa*) contains several phytochemicals with quercetin being an important and one of the most powerful flavonoids for protecting the body against reactive oxygen species [17]. Onion also contains organosulphur compounds, important for its good health with antimutagenic and antioxidant effects [18]. Tomato (*Lycopersicon esculentum*) is good a source of antioxidants, conferring it a highly nutritional vegetable. It is a veritable source of α-tocopherol, ascorbate, folates, carotenoids (lycopene and β-carotene) and some polyphenolic compounds [19].

Beef is a regular food eaten by most Nigerians- old and young, because of its nutritionally significant role of providing proteins, lipids, vitamins, minerals etc. The biochemical effects of beef processed by one of the indigenous methods were evaluated in this study. We examined the effect of compounds generated by the combined impact of heat, flame and smoke used in beef processing and the protective effects of crude extracts of tomatoes and onions.

2. MATERIALS AND METHODS

2.1 Preparation of Tomatoes, Onions and Beef

Fresh tomatoes and onion bulbs were bought from Ekeonuwa market in Owerri West Local Government Area (LGA), Imo State. These were washed and stored until used. The meat

was purchased at a market in Obinze Owerri West (LGA). 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 syntheses of PAHs. The fresh tomatoes and onions were washed with distilled water, homogenized and oven dried at reduced temperature (40ºC) to eliminate moisture. About 200 g of the dried tomatoes and onions were subjected to Soxhlet extraction with 180 ml of ethylacetate and methanol respectively. A gel-like residue was obtained after the elimination of the solvents.

2.2 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 were 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 antagonism. 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.

The experimental design of this work was in line with the guidelines on the care and wellbeing of research animals [20] and was approved by the Department of Biochemistry Ethics Committee.

2.3 Biochemical Studies

After 24 hours fast, the rats were sacrificed and blood samples were 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 [21] was used to determine serum aspartate amino-transferase (AST) and alanine amino transferase (ALT) activities. Serum alkaline phosphatase (ALP) was determined by the method of Englehardt et al. [22]. The method of Tietz [23] was used to determine total protein and the method of Doumas et al. [24] was used to determine the albumin concentration. Catalase was estimated by the method of Aebi [25]. Superoxide dismutase (SOD) was determined using the method of Xin et al. [26]. Glutathione concentration was determined according to King and Wootton [27]. Glutathione peroxidase (GPx) activity was estimated by the method described by Paglia and Valentine [28]. Lipid peroxidation was determined spectrophotometrically by measuring the concentration of malondialdehyde (MDA) by the method of Wallin et al. [29].

3. RESULTS

The effect of the content of the roasted meat and the protective effect of extracts of tomato and onion on liver function markers are shown below.

Fig. 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 fed the formulated diet and treated with the extracts. AST activity (Fig. 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). Fig. 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.

Fig. 3 also shows that the protein concentrations 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.

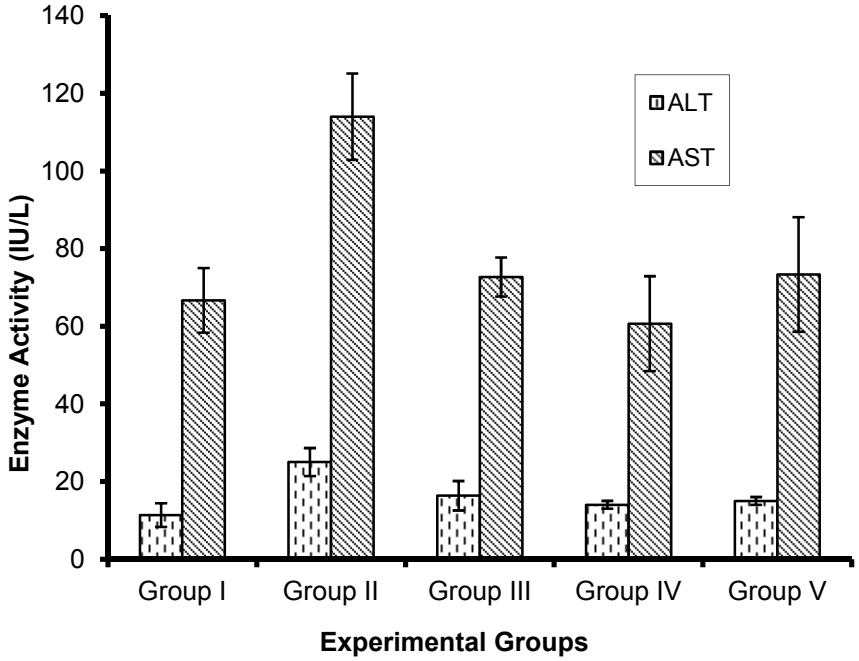

Fig. 1. Enzyme activities of alanine transaminase and aspartate transaminase

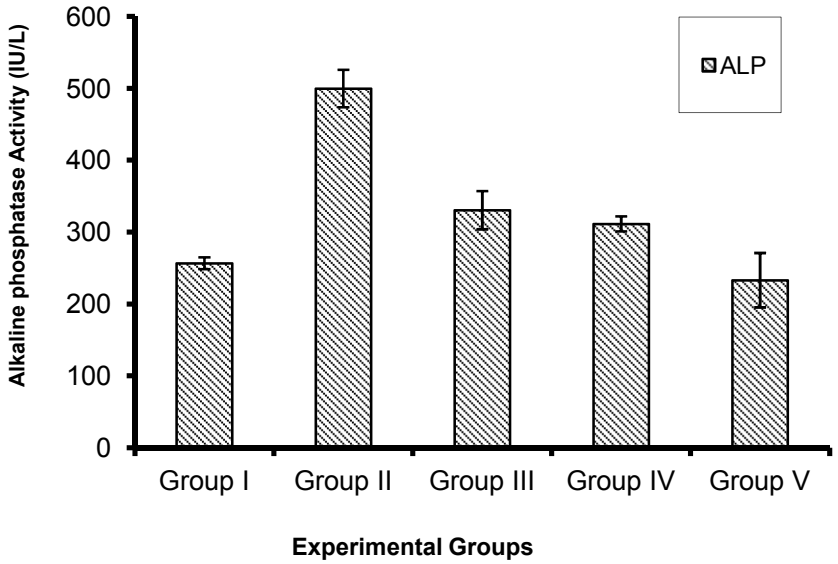

Fig. 2. Enzyme activity of alkaline phosphatase

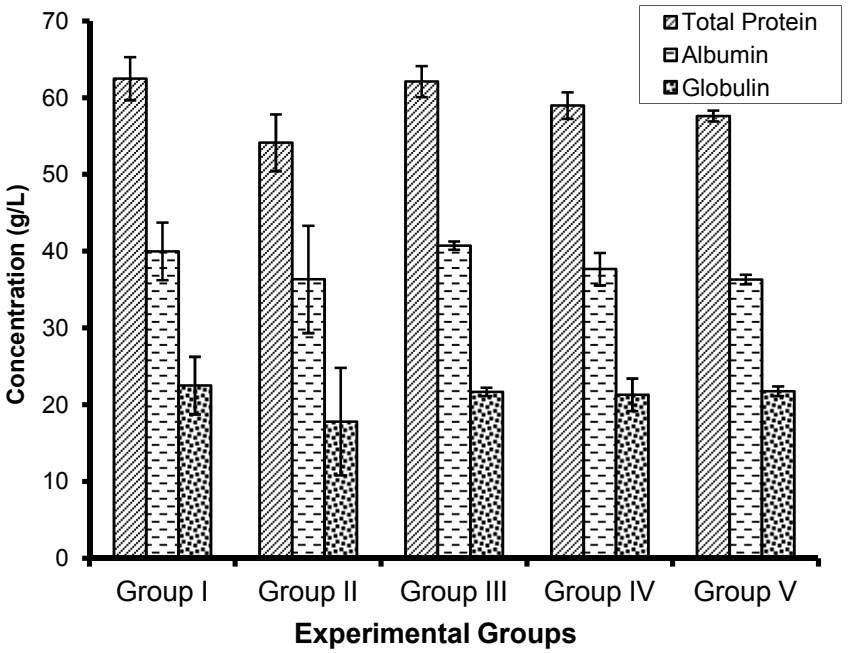

Fig. 3. Serum protein concentration of experimental rats

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^{cf}	76.67±5.86^{d}	33.33±4.16^{cf}
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 [30,31]. Some toxic substances such as PAH, HCA, heavy metals and other biochemically important constituents are generated during heat processing of beef using firewood [2,3]. These compounds can lead to oxidative liver damage [32]. 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 [33].

The reactive metabolites (epoxides) formed as a 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 [3], usually are metabolic activator of DNA adducts formation [14]. B(a)P disturbs the antioxidant defense system and can cause adverse effects due to redox cycling with semiquinone radical, thus increasing oxidative stress and DNA damage [34]. The biochemical impact of these chemical constituents can be appreciated by the values obtained for oxidative stress parameters. 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 activities of antioxidant enzymes 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 hepatoxicity due to epoxide formation that alkylates cellular protein and other macromolecules [35]. These metabolites are free radicals which could attack polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage [36]. Lipids are extremely susceptible to ROS, due to the peroxidation of polyunsaturated fatty acids abundant in cells [37]. The significantly increased (P<0.05) value of MDA (a lipid peroxidation product) in the rats fed 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 [38-40] and this may have caused the elevated liver enzymes in the blood [41] as shown by the results of this study. Under condition of severe oxidative stress, free radical generation may lead to protein modification and damage [42]. This may be attributed to the

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 tomatoes and onions on liver cells is indicated by the reduction in enzyme activities in extracellular milieu of rats treated with the extracts. These significant decrease ($P<0.05$) were within the enzyme activities observed in negative control (NC) rats. The crude extracts which are good sources of phytochemicals [43,44] may have contributed in restoration of the liver cell membrane permeability [45]. Some phytochemicals exert their effect through antioxidant mechanism [46-49], immune system modulation, carcinogen metabolism and metabolic pathways involving phase II drug-metabolizing enzymes [50]. Quercetin is a very active flavonoid extracted from onion and can protect cells against ROS induced damage [7]. Quercetin can scavenge free radicals through the inhibition of LDL oxidation [51,52], 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. Tomato is an important dietary source of antioxidants such as α-tocopherol and the carotenoids beta carotene, phytoene, and phytofluene. Tomato is also the main dietary source of lycopene, the most potent in vitro antioxidant among the carotenoids [53]. 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 [47,54]. 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 [55]. Quercetin ability to scavenge available free radicals reduces the tendency for its reaction with nitric oxide, resulting in less damage [46,56]. 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 peroxyl radicals by its addition to the long polyene chain [57]. This is in agreement with the findings of [58,59] 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 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 [60] who discovered that *Freeus racemoza* possesses potent hepatoprotective effects against carbon tetrachloride (CCl_4) induced hepatic damage in rats.

5. CONCLUSION

This study has shown that rats fed diets formulated with meats processed by traditional meat preparation (commonly called ‘suya’) induced reactive metabolites which could lead to hepatic damage and other debilitating consequences. Treatment with extracts of ethylacetate and methanol 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. To prevent oxidative stress related liver damage reduction in the exposure to endogenous and exogenous sources is recommended. This could be achieved 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. Also regular and adequate consumption of natural sources of antioxidants such as fruits and vegetables is encouraged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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