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Research Article

Tert-Butylhydroquinone Administration Alters Some Biochemical Markers in Male Wistar Rats Fed Kerosene Contaminated Diet

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Abstract

Tert-butylhydroquinone (TBHQ) and olive oil are commonly used antioxidant additives that are approved for human use by both the Food and Agriculture Organization and the World Health Organization (FAO/WHO). The tendency that TBHQ may cause substantial danger to public health with side effects is unclear. This study investigated the comparative effect of tert-butylhydroquinone and olive oil on some biochemical and haematological indices of Wistar rats fed kerosene contaminated diets. Twenty-six adult rats were divided into five groups; A, B, C, D and E. Groups A and B served as the positive controls treated with olive oil and tert-butylhydroquinone (0.5 mg/kg body weight + normal feed) respectively. Rats in groups C and D received diets contaminated with kerosene (4 ml/100g of feed) in addition to tert-butylhydroquinone (0.5 mg/kg body weight) and olive oil respectively. Rats in group E served as the negative control which were administered diets contaminated with kerosene without treatment. At the end of the treatment period, rats were sacrificed using ketamine anaesthesia. Results indicated that there was a significant increase ($p < 0.05$) in the concentrations of TG, VLDL-c and LDL-c in the group uncontaminated with kerosene and treated with tert-butylhydroquinone, and the group contaminated with kerosene and treated with olive oil when compared to the uncontaminated group that was treated with olive oil. Haemoglobin concentration increased significantly in the uncontaminated groups and the contaminated group treated with olive oil when compared to the contaminated group treated with tert-butylhydroquinone. The ALT activity of the group fed contaminated feed and treated with tert-butylhydroquinone and the group whose diets were not contaminated, increased significantly when compared to the group that was fed contaminated diet and treated with olive oil. AST activity of all the groups increased significantly when compared to the uncontaminated group administered olive oil. These suggest that olive oil may have an ameliorative impact on haematological indices due to its antioxidant properties while tert-butylhydroquinone could not attenuates some biochemical and haematological indices in rats fed kerosene contaminated diets.

Keywords: *Biochemical Markers; Haematological Indices; Tert-Butylhydroquinone; Kerosene*

Introduction

Kerosene is a middle distillate of the petroleum refining process, defined as the fraction of crude oil that boils between 145 and 300°C (U.S. Environmental Protection Agency [9]. Kerosene has been an important household fuel since the mid-19th century. In developed countries, its use has greatly declined because of electrification. However, in developing countries like Nigeria and Ghana, kerosene use for cooking, lighting and other domestic purposes is widespread. Due to the predominant of kerosene as a source of fuel in these developing countries (mostly in rural areas and some urban centers), regular exposure to its constituents and its combustion products is still high [2,20,37-39]. Kerosene has been proven to be capable of inducing oxidative stress via reduction of antioxidant defense system. Kerosene

combustion product like benzene has been associated with bone marrow degradation, pancytopenia and haematologic toxicity [4]. Acute inhalation and prolonged exposure to naphthalene is linked with pulmonary and renal injury [28]. In folkloric medicine, some people have obliviously misused kerosene in the expectancies of treating some ailments, thereby divulging themselves to these toxicants.

Healthy food choices and appropriate nutrition with safe foods are the most important factors in prolonging human life. Consumer concerns about food safety have grown in recent years as a result of increased public awareness and widespread consumption of processed foods, as well as changes in their lifestyle and eating habits [3,18]. The European Commission (EC) has emphasized reassessing the safety, pharmacokinetic, and toxicological characteristics of food additives due to the widespread use of food additives in processed foods and the high prevalence of chronic diseases [18]. Additives are added to foods for a variety of technological or sensory purposes, such as enhancing flavor, color, and texture, increasing shelf life, and reducing the risk of foodborne diseases [27]. These substances include flavorings, colorants, sweeteners, antimicrobials, and antioxidants [36]. Due to their multifunctional capabilities, antioxidants as food additives considerably reduce or delay oxidation and deterioration of the food products when added to foods at allowed amounts. Antioxidants are essential for reducing oxidative processes in both human bodies and food matrices. To stop food from spoiling and maintain the nutritional value of the food, food producers use a variety of synthetic antioxidants [10]. Additionally, food chemists and public health experts are interested in dietary antioxidants because they may help the body defend itself against harm caused by reactive oxygen species (ROS).

Olive oil contains phenolic substances including hydroxytyrosol and oleuropein, which are in charge of giving it both its distinctively pungent flavor and great durability. According to recent research, olive oil phenolics are the most potent antioxidants, both *in vitro* and *in vivo*, and they also have other powerful biological properties that may contribute to the Mediterranean diet's reported health benefits [41].

Olive oil phenolics may exert their antioxidant effects in addition to their free radical scavenger activity by encouraging the production of antioxidant molecules such HDL cholesterol and GSH-Px [21].

Tert-butylhydroquinone (TBHQ) is a patented potential oil-soluble antioxidant used as an effective additive in various products [16,24]. TBHQ is more efficient than other synthetic antioxidants in vegetable oils and animal fat [34]. In contrast, high quantities of TBHQ cause harmful effects on animals, such as inducing gastrointestinal tumors and damaging deoxyribonucleic acid (DNA) rings [26]. For instance, several studies have shown that TBHQ results in the development of 8-hydroxydeoxyguanosine (8-oxodG) in DNA due to ROS production such as superoxide anion and H_2O_2 [16]. Moreover, suppressing/increasing effects of TBHQ on gene expression can modify its cytotoxic and genotoxic effects on various cell lines [33]. In recent years, further studies have been suggested concerning multiple roles of the compound in public health [8]. However, the cytotoxic effects of TBHQs on various cells are still unclear. The present study soughts to investigate the impact of tert-butylhydroquinone on some biochemical and haematological indices of Wistar rats fed co with kerosene contaminated diets.

Materials and Methods

The kerosene used in this study was purchased from Ukoromi fuel station, No 49 New Airport road, Calabar, Cross River State, Nigeria.

Twenty five Wistar rats aged 10 -12 weeks and weighing 162 - 305 grams were purchased from the Department of Biochemistry, University of Calabar, Nigeria. These animals were maintained in standard conditions according to the procedure of the Animal Ethics Committee with approval number/code: CRUTECH/FBMS/IREC/2020-A1102 housed in well-ventilated standard cages and allowed to acclimatize to the environment and on Chikun feed (rat chow) and clean water for seven days. The development of experimental protocols and procedures were performed in accordance with the Public Health Service (PHS) Policy on Human Care and Use of Laboratory Animals.

Experimental design and sample collection [42]: A total of twenty five Male Wistar rats were divided into five different groups, based on their weights (n = 5). Their weights were taken before the commencement of the induction and at the end of the induction. Rats in groups C, D and E were administered feed contaminated with kerosene (4 ml/100g of feed) for twenty one days, to establish toxicity, while rats in groups A and B were given normal diets. At the end of the induction period, rats in groups A and D were administered olive oil, while rats in groups B and C were administered tert-butylhydroquinone (0.5 mg/kg body weight) for twenty eight days. During the administration period, rats in group E served as the negative control; rats were maintained on diets contaminated with kerosene and without treatment. All the rats received water ad libitum throughout the study period. During the experimental period, the body weight of each rat was assessed in grams after every seven days using a digital weighing balance. At the end of treatment period, all rats were anaesthetized using ketamine hydrochloride and sacrificed. Blood samples and relevant organs were harvested for the hematological and biochemical analyses.

Biochemical and haematological analyses: Activities of Aspartate aminotransferase (AST), Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) in serum were determined using Randox kits from Randox laboratories (55 Diamond roads, Crumlin, County Antrim BT29 4QY, United Kingdom). Randox kits were also used to assess serum triacylglycerol (TG), Furthermore; concentrations of low-density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were calculated using Friedwald formula [11]. Haematological parameters were analysed based on the methods adapted from [42].

Statistical analysis: The data are presented as mean ± SEM (n = 5). Data obtained were analysed using one-way ANOVA followed by least square difference (LSD) post-hoc comparison test to evaluate significant difference between the mean values of the experimental and control groups. Differences at $P < 0.05$ were regarded as significant. Graphpad prism version 7 and SPSS software package version 23.0 were used for the statistical analyses.

Results

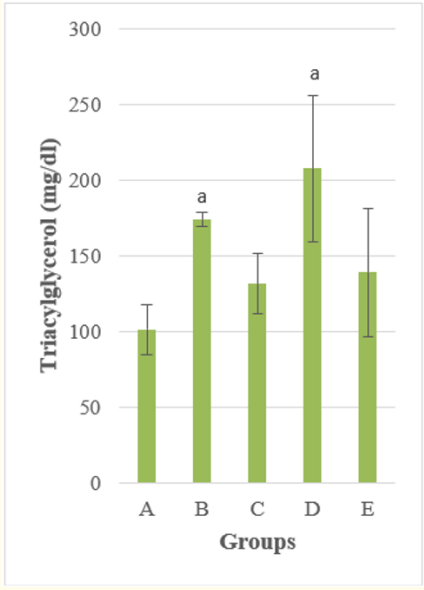

Figure 1: *Effect of tert-butylhydroquinone on serum triacylglycerol of rats fed kerosene contaminated feed.*

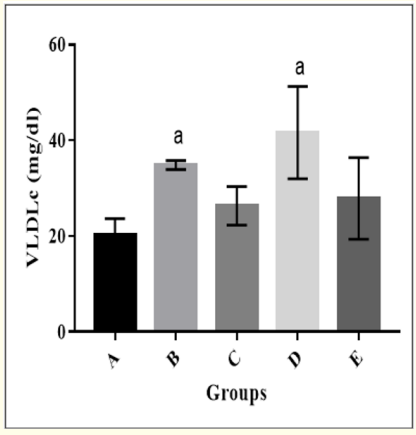

Figure 2: *Effect of tert-butylhydroquinone on serum VLDL-c of rats fed kerosene contaminated feed.*

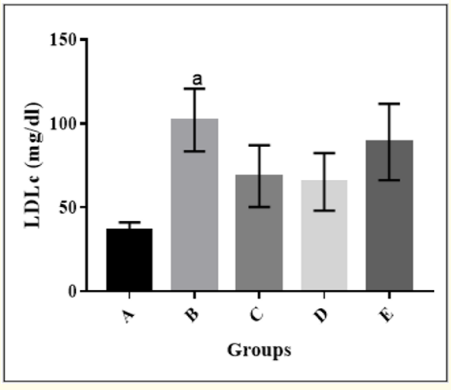

Figure 3: *Effect of tert-butylhydroquinone on serum LDL-c of rats fed kerosene contaminated feed.*

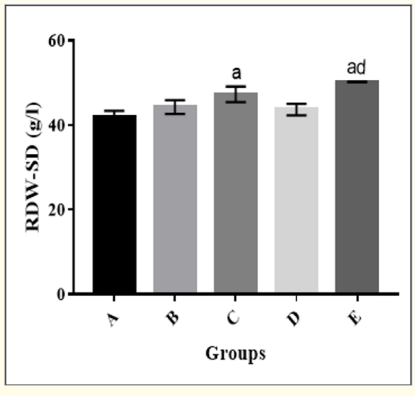

Figure 4: *Effect of tert-butylhydroquinone on serum RDW-SD of rats fed kerosene contaminated feed.*

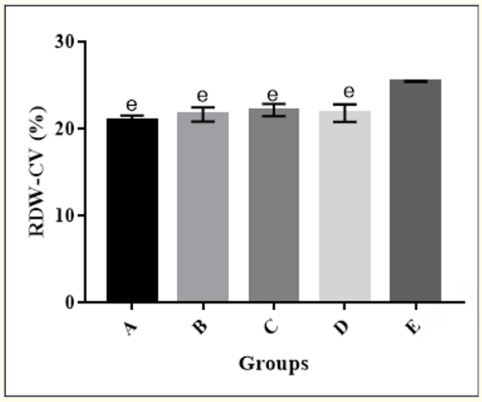

Figure 5: *Effect of tert-butylhydroquinone on RDW-CV of rats fed kerosene contaminated feed.*

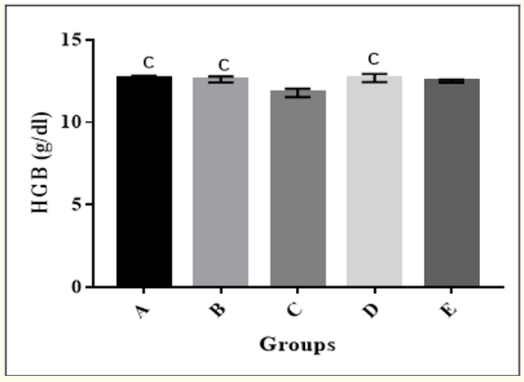

Figure 6: *Effect of tert-butylhydroquinone on HGB of rats fed kerosene contaminated feed.*

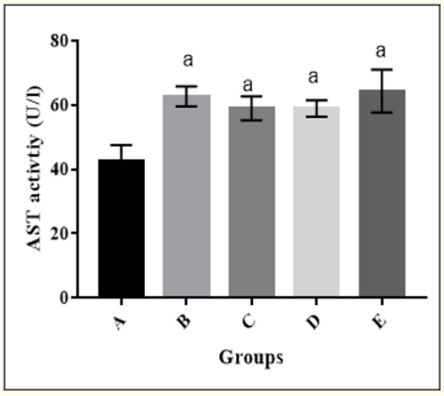

Figure 7: *Effect of tert-butylhydroquinone on serum AST of rats fed kerosene contaminated feed.*

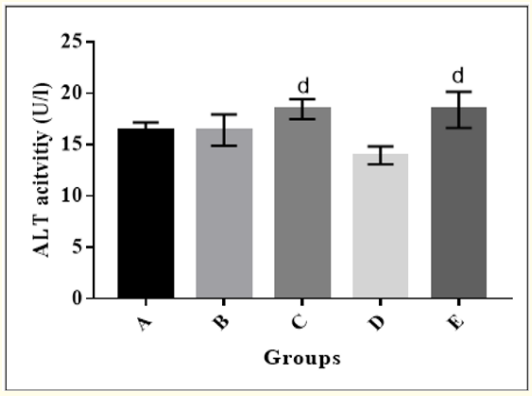

Figure 8: *Effect of tert-butylhydroquinone on serum ALT of rats fed kerosene contaminated feed.*

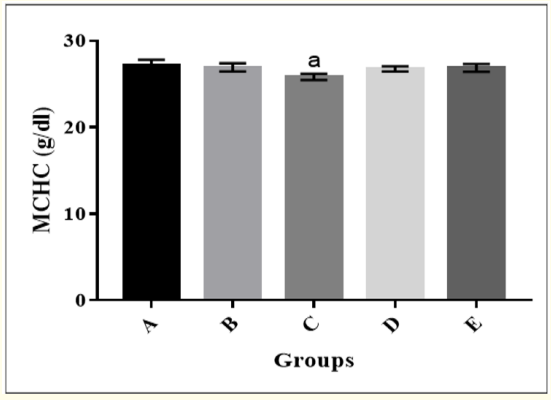

Figure 9: *Effect of tert-butylhydroquinone on MCHC of rats fed kerosene contaminated feed.*

Discussion

Although free radical production in a living system is not unrelated to physiologic process, its disproportionate production is a common phenomenon with xenobiotics exposure. Kerosene, a commonly available product in Nigeria, used as fuel for lighting and cooking purposes is an example of a xenobiotic that has been proven to be capable of inducing oxidative stress via reduction of antioxidant defense system and other metabolic insults to biological systems. Oxidative stress has been associated with the development of a wide range of diseases including Alzheimer's' disease [22]. Alongside the natural antioxidants, permitted concentrations of synthetic antioxidants have been demonstrated to exert protective effects against the oxidative stress-induced cytotoxicity. Olive oil and tert-butylhydroquinone have been reported to possess such antioxidant properties. In this study, the effect of tert-butylhydroquinone (tBHQ) and olive oil on some biochemical and haematological parameters of rats fed kerosene contaminated diet was evaluated.

The result of the serum lipids profile revealed that there was a significant increase in the concentration of TG in the group uncontaminated with kerosene and treated with tert-butylhydroquinone, and the group contaminated with kerosene and treated with olive oil

when compared to the uncontaminated group that was treated with olive oil. This is in disagreement with the findings of Kung-Woo., *et al*. (2012) who reported that tBHQ supplementation significantly lowered the plasma triglyceride and total cholesterol in mice. Olive oil has been reported to be the main source of fat of the Mediterranean diet which has been shown to be effective against oxidative stress associated diseases and also with ageing [23]. Here, the significant increase observed in the contaminated group treated with olive oil could be an indication of cardiac due to kerosene, and the administration of olive oil had no ameliorative effect. Studies have shown that when a fatty acid is damaged by free radicals, it becomes a free radical itself setting up a chain reaction of lipid peroxidation [12,40]. A similar trend was observed for VLDLc and LDLc. Elevated concentrations of LDL show a positive relationship with the severity of acute coronary events [14] and could be associated with tBHQ administration. Several studies have shown that TBHQ can result in the formation of 8-hydroxydeoxyguanosine in thymus DNA due to the production of ROS such as superoxide anion [17].

In this study, we found out that haemoglobin concentration increased significantly in the uncontaminated groups and the contaminated group treated with olive oil when compared to the contaminated group treated with tert-butylhydroquinone. Some components of the unsaponifiable fraction of olive oil, such as squalene, b-sitosterol, or triterpenes, have been shown to display antioxidant activity in experimental models [7]. The increased haemoglobin concentration observed in this study could be attributed to the reduction in the levels of free radicals triggered by kerosene administration that were subsequently reduced by components of olive oil. A seemingly reversed trend was observed in the Mean Corpuscular Haemoglobin Concentration (MCHC) as the contaminated group treated with tert-butylhydroquinone decreased significantly when compared to the uncontaminated group treated with olive oil. This observation agrees with the findings of [31] who opined that tBHQ decreased certain haematological indices in diazinon-induced oxidative stress in male Wistar rats. The Red Cell Distribution Width-Standard Deviation (RDW-SD) shows a significant decrease between contaminated group treated with tert-butylhydroquinone and untreated group when compared to the uncontaminated group treated with olive oil. The Red Cell Distribution Width-Coefficient of Variation (RDW-CV) of all the treated groups decreased significantly when compared with the untreated groups. According to previous experiments performed by [19], tBHQ, might also alter the erythrocyte membrane, eventually leading to partial or complete hemolysis and the formation of Heinz bodies.

The ALT activities of the group fed contaminated feed and treated with tert-butylhydroquinone and the group whose diets were not contaminated, increased significantly when compared to the group that was fed contaminated diet and treated with olive oil. The AST activity of all the groups increased significantly when compared to the uncontaminated group administered olive oil. Serum activity of hepatic transaminases is informative on hepatic health and pathology [5,13,15]. Increases in serum ALT and AST activities indicate hepatobiliary toxicity and hepatocyte injury [30]. The observed increases in these biomarker enzymes reported in this study agrees with previous exposure-related increases in serum hepatic transaminases [1], LDH activity and an increase in rat blood glucose level. [15] The significant decrease in the group treated with olive oil may be attributed to the antioxidant properties of olive oil.

Conclusion

Olive oil administration had an ameliorative impact on haematological indices due to its antioxidant properties while tert-butylhydroquinone altered some biochemical and haematological indices in Wistar rats fed kerosene contaminated feed.

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