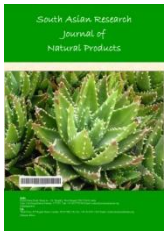

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Tert-Butylhydroquinone's Effect on Oxidative Stress Indices in High Fructose Challenged Rats' Skeletal Muscle

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Antioxidant and oxidative stress effects of prooxidants are generally dose-dependent, depending on the prooxidant species and cell type. However, the cellular response to oxidant challenge is a complicated interplay of events involving cellular expression of phase II detoxification enzymes and cellular metal metabolism. The study aimed to determine tert-butylhydroquinone's effect on oxidative stress indices in high fructose-challenged rats' skeletal muscles. A total of thirty (30) experimental rats were used in this study and weighed between 150 to 183 g. The rats were grouped into four (4) groups. Group, I (control) received distilled water and standard rat pellets. Group II (disease or fructose drinking group) received 21% of fructose drinking water (w/v) and standard rat pellets. Group III (Positive control or metformin group) received 21% of fructose drinking water and oral administration of metformin (300 mg/kg body weight daily), group IV (Test group): received 21% of fructose drinking water and 1% tert-butylhydroquinone feed. The rats were fed for 49 days (7 weeks). Preparation of skeletal muscle homogenates and assay of biochemical parameters were carried out. From our results, there was a non-significant elevation/decrease for

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all enzymes; thus, catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione-S-transferase (GST) activity in the skeletal muscles of rats at (P>0.05) when compared to the control. MDA and GST activity expressed upregulation, while SOD and CAT expressed downregulation. TBHQ can ameliorate insulin resistance in the skeletal muscle of high fructose challenged rats, providing evidence for TBHQ's clinical use to treat T2DM.

Keywords: High-fructose; oxidative stress; tert-butylhydroquinone's and type 2 diabetes.

1. INTRODUCTION

"Insulin resistance, type 2 diabetes, dyslipidemia, and hypertension are all on the rise worldwide, and this trend is due mainly to lifestyle decisions" [1]. "These metabolic abnormalities have been linked to unhealthy eating habits, such as a high intake of simple carbohydrates" [1]. "Consumption of soft drinks, particularly carbonated beverages, or fructose as a sweetener in beverages and processed foods (either as high fructose corn syrup or sucrose) contributes to excessive dietary carbohydrate consumption. As a sweetener in beverages, soft drinks, and carbonated drinks, fructose has increased worldwide, including Nigeria. It has been linked to developing metabolic syndromes (MetS) components like insulin resistance, type 2 diabetes, dyslipidemia, and hypertension in people living in these areas" [1].

"Oxidative stress has a role in the aetiology of several clinical conditions, including hypertension" [2], coronary heart disease [3], and type 1 and type-2 diabetes mellitus [2,4]. Some ROS are very hazardous, and they are swiftly detoxicated to more minor damaging metabolites by various cell processes, both enzymatic and non-enzymatic [5]. **"**However, high levels of muscular activity result in a significant increase in the creation of reactive oxygen species (ROS). Due to an unpaired electron, these unstable molecules and ions contain oxygen and are particularly reactive" [6]. "The free radicals superoxide, peroxide, hydroxyl radicals and other highly reactive oxidants such as singlet oxygen and hypochlorous acid are oxygen intermediates. They can be extremely harmful because they induce oxidation reactions with other molecules such as proteins, lipids, and DNA" [6]. Several researchers have found that excessive fructose consumption promotes oxidative stress by disrupting the equilibrium between free radical generation and endogenous antioxidant status, resulting in cardiovascular abnormalities and problems [6]. However, this has necessitated the search for potent and cost-effective synthetic antioxidants.

Polyphenols are secondary plant metabolites with one or more hydroxyl groups linked to the benzene ring's ortho, meta, or para locations. They have been proven to have antioxidant, anti-inflammatory, anti-hypertriglyceridemic, antidiabetic, and anti-hypercholesterolemic qualities, among other things [7,8].

Tert-butyl hydroquinone (TBHQ) is among edible synthetic antioxidants, primarily used as a food additive for unsaturated vegetable oils [9]. "TBHQ are phenolic and organic aromatic chemicals generated from hydroquinone" [9]. "TBHQ is a potent antioxidant added to unsaturated vegetable oils and various edible animal fats. TBHQ is thought to work primarily by causing nuclear translocation of the transcription factor nuclear factor erythroid 2-related factor 2 (NF-E2), which then regulates the gene expression of antioxidant enzymes such as heme oxygenase-1 and glutathione S-transferase" [10], "NAD(P)H quinone oxidoreductase, and heme-oxygenase-1" [10].

Given the metabolic consequences of high fructose consumption (one of which is oxidative stress) and dietary polyphenols' therapeutic and antioxidant potentials, including tertbutyl hydroquinone, the current study looked at tert-butylhydroquinone's effect on oxidative stress indices in high fructose challenged rats' skeletal muscle.

2. MATERIALS AND METHODS

2.1 Chemical/Reagents

All the chemicals used in this study were of analytical grade and products of May and Baker (England) and Merck and Darmstadt (Germany). The reagents used for all the assays were already made/commercial-grade kits, products of Randox, QCA (USA) and Biosystem Reagents and Instruments, Spain.

2.2 Animal Protocol

For this investigation, 30 Albino Wistar rats weighing 150 to 183 g were acquired from an

animal house at the University of Nigeria Nsukka. Rats were kept in plastic cages in a well-ventilated room, fed rat food and water ad libitum, and exposed to a natural 12-hour light/12-hour dark photoperiod. All animals were treated humanely per the guidelines in the US National Academy of Science's (NAS) Guide for the Care and Use of Laboratory Animals, which the US National Institute of Health issued.

2.3 Experimental Design

Following a two-weeks acclimatization period, the rats were divided into four groups of four rats per group as follows:

Group 1 (Control): received distilled water and standard rat pellets

Group 2 (Disease or fructose drinking group): received 21% of fructose drinking water (w/v) and standard rat pellets

Group 3 (Positive control or metformin group): received 21% of fructose drinking water and oral administration of metformin (300 mg/kg body weight daily)

Group 4 (test group): received 21% of fructose drinking water and 1% tert-butylhydroquinone feed [11, 12].

“For the preparation of tert-butylhydroquinone feeds, the rat pellets were powdered in a grinder and dry mixed with 1% tert-butylhydroquinone (w/w). Distilled water was added to the powder. The setup was thoroughly mixed, reshaped into food pellets and oven at 50°C for 24 h. This dose of tert-butylhydroquinone has been widely used in animal models” [11,12]. At the end of 7 weeks, the rats fasted overnight, and the next day, blood was harvested from their femoral vein under mild anaesthesia with chloroform.

2.4 Preparation of Skeletal Muscle Homogenates

1 g of the muscle from each rat was homogenized in Tris–HCl buffer (pH 7.4). The homogenate (10%) was centrifuged at 2,000 x g for 20 minutes, and the aliquot was used to measure malondialdehyde, catalase, Glutathione-S-transferase, and superoxide dismutase activities in the rats' skeletal muscles.

2.5 Assay of Biochemical Parameters

“Lipid peroxidation was assayed by determining the malondialdehyde (MDA) concentrations using the method of” Chatterjee et al. [13]. “Catalase activity was measured using the method of Goth” [14]. **“**Glutathione-S-transferase activity was determined following” Habig et al. [15] method. **“**Superoxide dismutase activity was determined using the Nitro Tetrazolium Blue reduction method, and results were expressed as units/mg of protein. One unit of enzyme activity was defined as the amount of enzyme required to reduce nitro tetrazolium blue by 50%” [16].

3. RESULTS

Fig. 1 shows the findings of the injected superoxide dismutase activities of the rats. As seen in Figure, the disease group's SOD activity decreased non-significantly ($P<0.05$) compared to the control group. The SOD activities of the high fructose drinking rats were not substantially increased ($P>0.05$) by supplementation with tBHQ compared to the disease group.

Fig. 2 shows the findings of the catalase activities of the investigated rats. As indicated in the Figure, the disease group's catalase activity was non-significantly decreased ($P<0.05$) than the control group's catalase activity, and the tert-butylhydroxylquinone group's catalase activity was non-significantly increased ($P>0.05$) than the disease group's catalase activity.

The results of the glutathione-S-transferase activities of the rats that were studied are shown in Fig. 3. As presented in the Figure, there were no significant differences ($P>0.05$) in the glutathione-S-transferase activities of the rats in the respective groups.

Fig. 4 shows the findings of the malondialdehyde concentrations in the rats investigated. As presented in the Figure, there were no significant differences ($P>0.05$) in the malondialdehyde concentrations of all the rats in the respective groups.

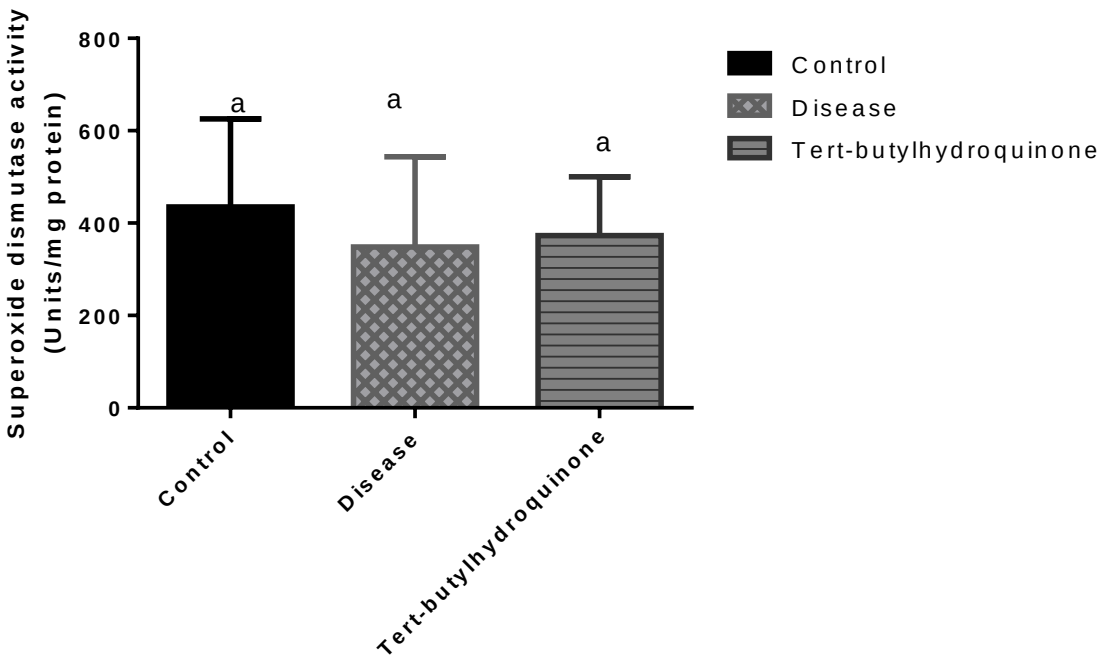

Fig. 1. Superoxide dismutase activity in the skeletal muscles of rats. Data are reported as means ± standard errors. [a]Means with the same superscript letters are not significantly different (P>0.05)

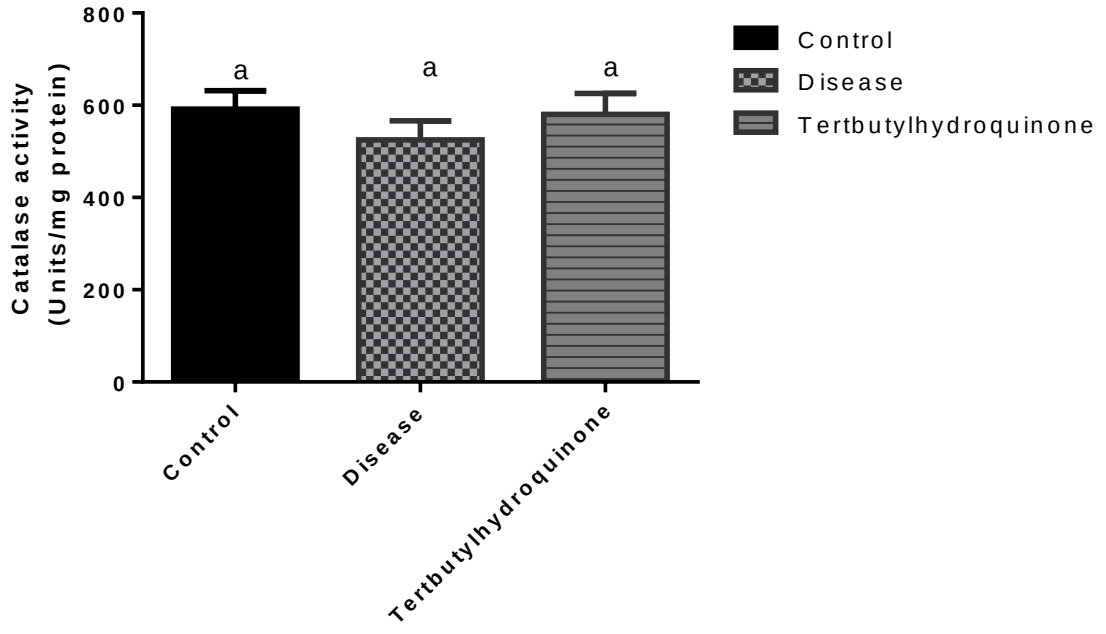

Fig. 2. Catalase activity in the skeletal muscles of rats. Data are reported as means ± standard errors. [a]Means with the same superscript letters are not significantly different (P>0.05)

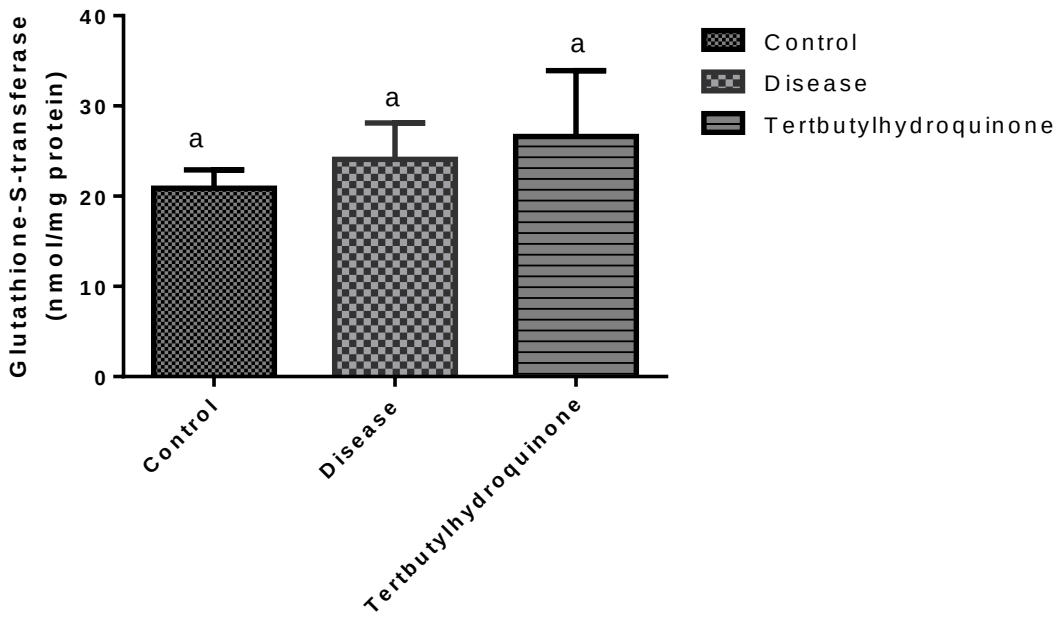

Fig. 3. Glutathione-S-transferase activity in the skeletal muscles of rats. Data are reported as means ± standard errors. [a]Means with the same superscript letters are not significantly different (P>0.05)

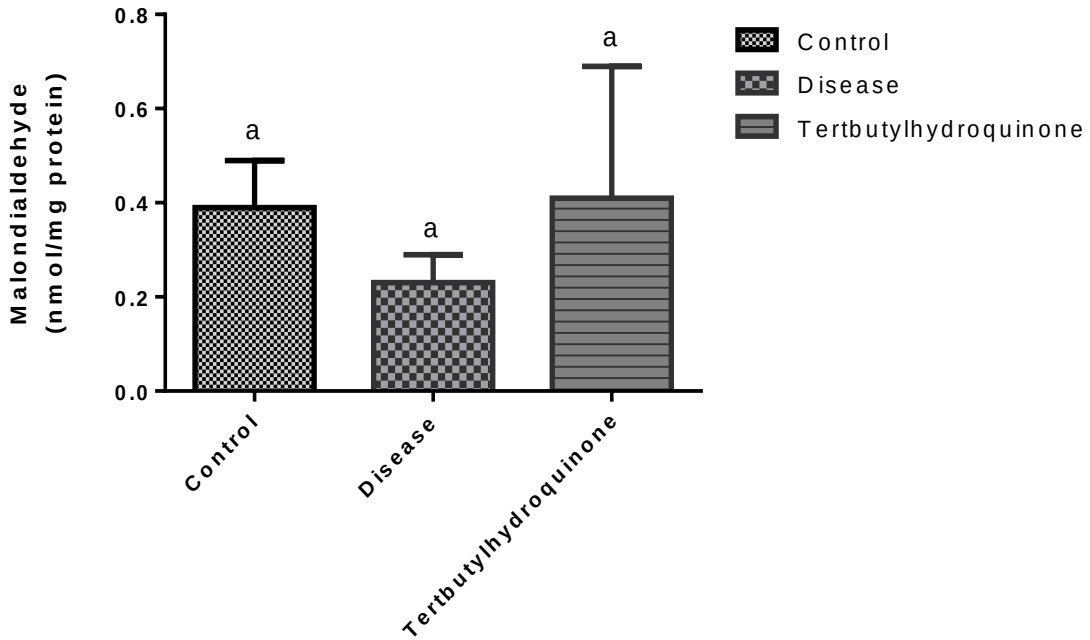

Fig. 4. Malondialdehyde concentration in the skeletal muscles of rats. Data are reported as means ± standard errors. [a]Means with the same superscript letters are not significantly different (P>0.05)

4. DISCUSSION

"Tert-butyl hydroquinone (TBHQ) is a hydroquinone-derived organic aromatic molecule. TBHQ is an antioxidant added to unsaturated vegetable oils and various edible animal fats. TBHQ can cause the transcription factor NF-E2-related factor 2 (Nrf2) to translocate to the nucleus, where it regulates the expression of vintages that code for cytoprotective phase 2 antioxidant proteins like glutathione-S-transferase, NAD(P)H quinone oxidoreductase, and heme-oxygenase" [10].

"The superoxide anion produced by NADPH oxidase is converted to oxygen and hydrogen peroxide by superoxide dismutase" [17]. Hydrolysis of hydrogen peroxide created by SOD to water and oxygen is catalyzed by glutathione peroxidase, and any hydrogen peroxide not broken down by glutathione peroxidase is broken down further catalase. When glutathione peroxidase and catalase are lacking, the released hydrogen peroxide accumulates, causing lipid peroxidation in the membrane and the production of malondialdehyde, a lipid peroxidation end product [17]. "Glutathione-S-transferase is essential in detoxifying ROS by conjugating with GSH and protecting tissues from oxidative stress" [18,17].

Our study found no oxidative stress in the skeletal muscles of the high fructose drinking rats, as seen from the non-significant effect of high fructose drinking water on the antioxidant enzymes and malondialdehyde concentrations of the disease group. This effect of fructose administration did not surprising, considering that the skeletal muscle is not the primary organ of fructose metabolism. Additionally, our study revealed that supplementing metformin and tBHQ to the high fructose drinking rats did not affect the activities and concentrations of the antioxidant enzymes and malondialdehyde.

5. CONCLUSIONS

The findings demonstrated that increased drinking fructose-induced skeletal muscle changes might not be linked to skeletal, muscular oxidative stress as reported in the literature. However, it is recommended that more research should be conducted to understand the molecular basis for the skeletal muscle changes caused by excessive fructose consumption.

ETHICAL APPROVAL

Animal Ethics committee approval has been taken to carry out this study

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper

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