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Quercetin, a natural phytochemical and antioxidant protects against sodium azide-induced hepatic and splenic oxidative stress in rats

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ABSTRACT

Introduction: The study investigated the possible ameliorative potentials of quercetin, a flavonoid, in the treatment of sodium azide (NaN_3)-induced oxidative stress in wistar rats. **Methods:** Oxidative stress was assessed by determining the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), as well as levels of reduced glutathione (GSH) and malondialdehyde (MDA). **Results and Conclusions:** No significant difference was seen in the relative liver weights in all groups, while quercertin treatments significantly reduced relative spleen weight. NaN_3-induced hepatotoxicity, as marked by elevated activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (GGT), as well as hepatic and splenic lipid peroxidation, as marked by elevated MDA concentrations were significantly reduced by quercetin treatments. Increases in hepatic CAT activity, as well as hepatic and splenic GSH levels induced by NaN_3, were significantly reduced following quercetin treatments. Quercetin treatment did not have any effect on splenic CAT and GST activities, but significantly increased hepatic GST activity compared to NaN_3 group. Decreased splenic SOD and GPx activities, as well as hepatic GPx activity following NaN_3 treatment were significantly increased by quercetin treatment. Tissue anti-oxidative potentials of quercetin may therefore be harnessed against NaN_3-induced hepatic and splenic oxidative stress.

KEY WORDS: Hepatotoxicity; Oxidative Damage; Flavonoid; Phytomedicine

INTRODUCTION

Sodium azide (NaN_3) is a colourless, crystalline solid, manufactured as a very fine powder [1]. Crystalline sodium azide is explosive. It decomposes on heating, emitting toxic fumes [2]. It is used as a preservative in aqueous laboratory reagents, as a gas generating chemical in automobile air bags and seat ejectors of jet planes. Results of *in vitro* studies using tissue homogenates and *in vivo* studies in rats showed that azide is metabolized completely mainly in the liver [3], to form nitrogen oxide, a toxic metabolite [4].

With increasing health hazards and problems emanating from exposure to lethal chemicals, medicinal plant research has grown abruptly during the last two decades. Medicinal properties of plants and plant derivatives have been exploited in the treatment of different ailments. One of such is quercetin.

Quercetin (3, 3', 4', 5, 7-pentahydroxy flavones), a flavonoid, is a polyphenolic compound that are found exclusively in plants. These compounds are able to elicit various biological and pharmacological activities in animal cells [5]. Quercetin, abundant flavonoids in human diet, is a strong reactive oxygen species (ROS) scavenger and good metal chelator [6]. It is rich in phenolic hydroxyl groups that have strong antioxidant activity [7,8]. High concentrations of quercetin are found in apples, onions, potatoes, broccoli, tea, soybeans, and red wine. Quercetin has very potent antioxidant and cytoprotective effects in preventing endothelial apoptosis caused by oxidants [9,10]. Quercetin has been used to treat hepatotoxicity, liver fibrosis, and many diseases [11-13]. In Western diets, the richest sources of quercetin are onions (347 mg/kg), apples (36 mg/kg), tea (20 mg/kg) and red wine (11 mg/kg) [14].

Objectives

1. There is still a dearth of information on treatment of NaN_3-induced tissue oxidative stress. In view of these, this study investigated the protection of quercetin against NaN_3-induced hepatic and splenic oxidative stress in male wistar rats.

MATERIALS AND METHODS

Sodium azide (NaN_3) and quercetin (purity ≥ 98 %) used in this study were of analytical grade, product of Sigma Chemical Co., Saint Louis, MO, USA. AST, ALT, and GGT kits are product of Cypress Diagnostics, Langdorp, Belgium.

Twenty (20) male wistar albino rats with an average weight of 150 g used in this study were obtained from the animal house of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria. They were housed in steel metal cages in the animal house of our department and were served food and water *ad libitum*. Permission to use the animals was approved by the Institution's Animal Ethical Committee.

Dose of 100 mg/kg quercetin was selected based on the literature survey of previous study [15], while doses of intraperitoneal injection between 15 and 150 mg/kg of NaN_3 into rats caused symptoms such as tremor, salivation, paralysis, convulsions, bradycardia or dyspnoea and later death [16,17].

Rats were randomly divided into four groups (I–IV) of five animals each. Group I animals serve as normal control, group II animals were administered a single intraperitoneal dose of 20 mg/kg NaN_3 for 48 hours, group III animals were also administered same as group II, but treated with 100 mg/kg quercetin orally for seven days, while group IV animals were administered 100 mg/kg quercetin only for seven days.

At the end of the experimental period, the animals were sacrificed. They were handled and used in accordance with the international guide for the care and use of laboratory animals [18]. Blood was collected via abdominal artery into clean heparinized tubes followed by centrifugation at 3000 rpm for 10 minutes. Plasma was separated into clean 1 ml eppendorf tubes, and stored at - 18°C until when used. Liver and spleen were also harvested, washed in ice-cold saline (0.9 % w/v) solution, blotted dry, and then weighed, after which they were suspended in ice-cold 0.1 M phosphate buffer (pH 7.4) and homogenized. Homogenization is then followed by centrifugation at 5000 rpm for 10 minutes. The homogenate was then used immediately for analysis of biochemical parameters.

Plasma activities of AST, ALT, and GGT were determined according to the methods described in Cypress Diagnostics Kits, Langdorp, Belgium. Activity of catalase was determined by the method of [19]. Sample (0.1 ml) was added to quartz cuvette containing 1.9 ml of 10 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Hepatic and splenic SOD activities were determined by the method of [20]. The method is based on the ability of superoxide dismutase to inhibit autooxidation of adrenaline to adrenochrome at alkaline pH. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of adrenaline auto-oxidation. Hepatic and splenic GPx activities were determined by the method of [21] where the color developed was read at 412 nm. Hepatic and splenic activities of glutathione S-transferase (GST) were determined by the method of [22] based on enzyme-catalysed condensation of glutathione with the model substrate, 1-chloro-2,4-dinitrobenzene. The product formed (2,4-dinitrophenylglutathione) absorbs light at 340 nm. Hepatic and splenic MDA concentrations, a marker of lipid peroxidation (LPO) were determined by the method of [23]. In this procedure, 1.0 ml of the supernatant was added to 2 ml of trichloroacetic acid–thiobarbituric acid–hydrochloric acid (TCA/TBA/HCl) (1:1:1 ratio) reagent, boiled at 100 °C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532 nm against blank. MDA concentration was calculated using the molar extinction coefficient for MDA-TBA complex of $1.55 \times 10^6\ M^{-1}cm^{-1}$. Hepatic and splenic reduced glutathione (GSH) levels were determined by the method of [24] where the color developed was read at 412 nm. Tissues concentrations of total protein were determined by the method of [25]. Data were analyzed by one-way analysis of variance (ANOVA), followed by least significant difference (LSD) to test for significant differences among the groups of rats using Statistical Package for Social Sciences program version 17.0. Data were expressed as mean ± standard error of mean. P values less than 0.05 were considered statistically significant.

RESULTS

Our results revealed no significant difference ($p > 0.05$) in relative liver weights in all the groups (Table 1), while a significant ($p < 0.05$) increase in relative spleen weight was recorded in NaN_3 only group when compared with the quercetin treated groups (Table 1).

Table 1. Relative organ weights of quercetin treatments in NaN_3-induced toxicity in rats.

GROUPS	Relative liver weight (%)	Relative Spleen weight (%)
Control	3.28 ± 0.08[a]	0.37 ± 0.03[a]
NaN_3	2.97 ± 0.09[a]	0.43 ± 0.04[b]
NaN_3 + Quercetin	3.21 ± 0.26[a]	0.30 ± 0.01[a]
Quercetin	2.98 ± 0.39[a]	0.28 ± 0.04[a]

Values are expressed as Mean ± Standard Error of Mean (n = 5). Values labeled with different superscript are statistically significant ($p < 0.05$). Relative organ (kidney, liver, spleen, testes) weight = (organ weight/final body weight) x 100

In NaN_3 treated group, the activities of AST, ALT, and GGT increased significantly ($p < 0.05$) compared to normal control group (Table 2). Quercetin treatment significantly ($p < 0.05$) treated the NaN_3 azide-induced hepatotoxicity by decreasing the plasma activities of the enzymes (Table 2).

Table 2. Hepatoprotection of quercetin in NaN_3-induced liver damage in rats

GROUPS	AST (U/L)	ALT (U/L)	GGT (U/L)
Control	5.37 ± 1.30[a]	2.45 ± 0.56[a]	1.19 ± 0.35[a]
NaN_3	38.50 ± 1.70[b]	11.67 ± 1.84[b]	6.35 ± 0.74[b]
NaN_3 + Quercetin	6.56 ± 1.05[a]	2.48 ± 0.28[a]	2.18 ± 0.12[a]
Quercetin	6.88 ± 1.41[a]	1.40 ± 0.40[a]	2.06 ± 0.34[a]

Values are expressed as Mean ± Standard Error of Mean (n = 5). Values labeled with different superscript are statistically significant ($p < 0.05$)

There was a significant ($p < 0.05$) increase in the concentrations of liver and spleen MDA in NaN_3-administered rats compared to normal control, but treatment with quercetin significantly ($p < 0.05$) decreased the MDA concentrations in both tissues to levels comparable to control (Fig. 1).

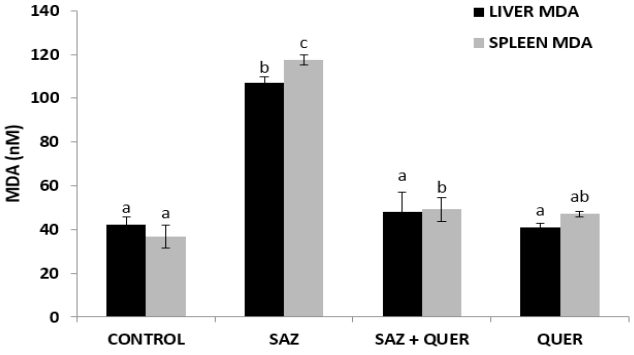

Figure 1. Effects of quercetin in NaN_3-induced hepatic and splenic lipid peroxidation in rats. Bars are mean values of MDA ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN_3 = sodium azide; QUER = quercetin

A significant ($p < 0.05$) increase in hepatic and splenic GSH levels in NaN_3-administered rats compared to normal control rats was recorded, but treatment with quercetin significantly ($p < 0.05$) decreased the GSH concentrations in both tissues to levels comparable to control (Fig. 2).

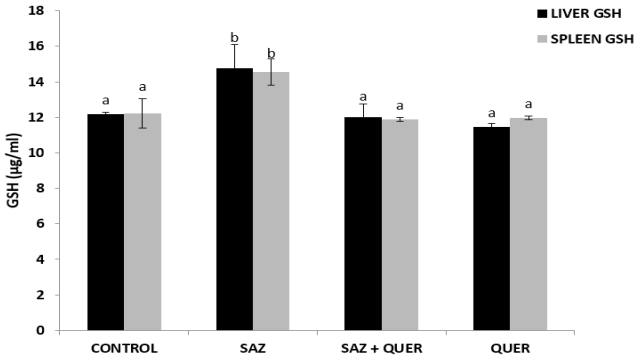

Figure 2. Effects of quercetin on hepatic and splenic GSH levels in NaN3-treated rats. Bars are mean levels of GSH ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN3= sodium azide; QUER = quercetin

For hepatic GST activity, a significant ($p < 0.05$) decrease was seen in NaN_3 only administered rats compared to normal control rats, but the was restored by quercetin treatments, while the significant increase ($p < 0.05$) in splenic GST activity in NaN_3 group compared to normal control rats, was not significantly ($p > 0.05$) reduced by quercetin treatments (Fig. 3). Splenic SOD (Fig. 4) and GPx (Fig. 5) activities, as well as hepatic GPx activity, were significantly ($p < 0.05$) reduced in NaN_3 administered rats compared to control rats, but hepatic SOD activity was not significantly ($p > 0.05$) reduced compared to control (Fig. 4). Quercetin treatment was able to significantly ($p < 0.05$) raise the activities of SOD (Fig. 4) and GPx (Fig. 5) in both tissues, to levels comparable to control. Lastly, hepatic CAT activity (Fig. 6) was significantly increased ($p < 0.05$) following NaN_3 administration compared to control, but was also significantly reduced ($p < 0.05$) after treatment with quercetin. Quercetin treatment did not have any significant effect ($p > 0.05$) on NaN_3-induced decrease in splenic CAT activity compared to control (Fig. 6).

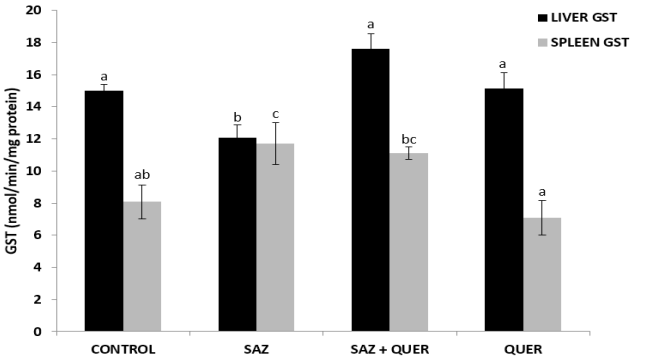

Figure 3. Effects of quercetin on hepatic and splenic GST activities in NaN3-treated rats. Bars are mean activities of GST ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN3 = sodium azide; QUER = quercetin

DISCUSSION

Generally, studies on flavonoids are widely geared to focus on quercetin [26]. Quercetin sources are largely abundant and easy to extract, isolate and detect [27]. It has multiple beneficial effects [28,29], among which are antioxidative [30-33], anti-inflammation [34], and anti-apoptotic [35,36].

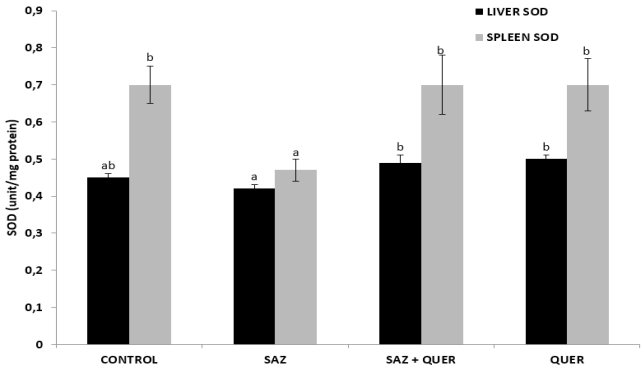

Figure 4. Effects of quercetin on hepatic and splenic SOD activities in NaN3-treated rats. Bars are mean activities of SOD ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN3 = sodium azide; QUER = quercetin

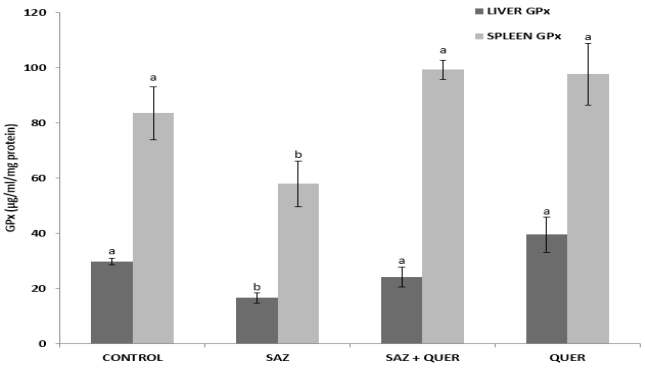

Figure 5. Effects of quercetin on hepatic and splenic GPx activities in NaN3-treated rats. Bars are mean activities of GPx ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN3= sodium azide; QUER = quercetin

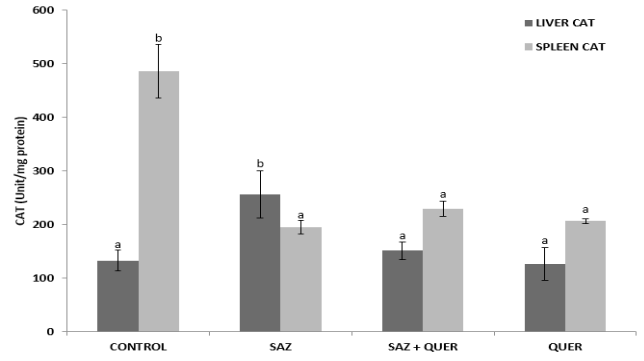

Figure 6. Effects of quercetin on hepatic and splenic CAT activities in NaN3-treated rats. Bars are mean activities of CAT ± SEM; n = 5. Bars with different letters are significantly different ($p < 0.05$). SAZ or NaN3 = sodium azide; QUER = quercetin

In the present study, the significant increase ($p < 0.05$) in relative spleen weight by NaN_3, compared to quercetin treated rats may be as a result of its ability to bind to iron in the center of the porphyrin ring of hemoglobin [37], as the spleen is known to be the site for the production and removal of red cells.

AST, ALT, ALP and GGT activities are used in the diagnosis of hepatic injuries and diseases. The activities of these enzymes are known to increase in blood as a result of hepatic damage or injury [38]. In this study, the hepatotoxicity of azide [4] was corroborated by marked elevation in activities of ALT, AST, and GGT. Reduction in the activities of these hepatic function marker enzymes, following quercetin treatment indicated the hepatoprotective potential of the flavonoid [39,40].

Free radicals are continuously produced in the body, which may result into cell damage. It is therefore necessary for tissues to be protected against this oxidative injury through intracellular and extracellular antioxidants [41]. Antioxidants delay or inhibit oxidative damage to target molecules [42,43]. Measurement of thiobarbituric acid (TBARS) is mostly used to monitor lipid peroxidation and indirectly, oxidative stress *in vitro* and *in vivo* [44]. The lipid oxidation causes disruption of the bilayer and cell integrity accompanied by leakage of cellular content from the damaged organ into the blood stream [45]. The significant increase ($p < 0.05$) in hepatic and splenic levels of MDA by NaN_3 administration may be as a result of free radicals attack on the electron-rich membrane components, while the significant decrease in the levels of MDA in both tissues after quercetin treatments may be as a result of its antioxidant and free radical scavenging ability, that may have spared the cell membrane components, by donating its rich electron to the unstable, unpaired, and ravaging free radicals. Our findings is also supported by the reports of [39] who used quercetin to ameliorate systemic oxidative stress in cirrhotic rats, and Alkhamees [46], who used quercetin to attenuate testicular damage and oxidative stress in streptozotocin-induced diabetic rats.

Oxidative stress is characterized by increase in the levels of free radicals due to insufficient antioxidant defense [47]. Defensive responses of organisms to oxidative stress include the utilization of endogenous antioxidant enzyme systems, lipid soluble and water soluble antioxidant molecules and phytochemicals. The antioxidant enzymes such as SOD, CAT and GPx are the main enzymes that act as defenses, as well as non-enzymatic antioxidants such as GSH [48]. They protect against the destructive effects of ROS. The significant increase ($p < 0.05$) in the concentrations of hepatic and splenic GSH following NaN_3 exposure may be due to the prompt and adaptive response of the cells to NaN_3-induced free radical generation, and probably an efficient reduction of oxidized glutathione (GSSH) back to the reduced state, in an attempt to mop the generated free radicals. SOD is the cell's first line of defense against oxidative stress [49], and is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to hydrogen peroxide (H_2O_2) and O_2.

In this study, superoxide radicals may have been generated as a result of NaN_3 administration, which resulted to significant decrease ($p < 0.05$) in the activities of hepatic and splenic SOD activities; also in an attempt to scavenge the radical [50]. CAT and GPx are responsible for the catalytic decomposition of H_2O_2 to molecular oxygen and water [48]. Also, the significant decrease in the activities of hepatic and splenic GPx may explain the results obtained for GSH levels in NaN_3 exposed group. The depletion of GPx by NaN_3 administration may be responsible for the significant increase ($p < 0.05$) in the level of GSH. As GPx detoxifies H_2O_2, there is a concomitant oxidation of GSH to GSSH, and then back to GSH, by glutathione reductase. Lastly, the significant increase ($p < 0.05$) in hepatic catalase activity by NaN_3 exposure may be attributed to the joint effort of CAT and GPx in detoxifying H_2O_2 [50].

The amelioration of NaN_3-induced tissue oxidative stress recorded in the study by quercetin treatments may signifies the anti-oxidative and tissue-protective potentials of the flavonoid, which may have spared and restored back the normal levels and activities of the endogenous antioxidant systems. The protective effects by quercetin treatment against tissue oxidative stress obtained from this study are corroborated by findings of [39,40,46,51-54].

In conclusion, tissue anti-oxidative potentials of quercetin may be harnessed against NaN_3-induced hepatic and splenic oxidative stress.

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