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Research paper

Thyroid and reproductive hormones disruption as well as kallikrein-3 level in dimethyl nitrosamine-induced toxicity: Effects of ascorbate treatment in male wistar rats

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Abstract

Information on dimethyl nitrosamine (DMN)-induced toxicity on endocrine functions is still scanty. This study therefore investigated the outcomes of DMN-induced toxicity on endocrine (thyroid and reproductive) functions, as well as kallikrein-3 level, and effects of ascorbate treatments in male wistar rats. Thirty animals divided into six groups of five rats each were used. Group I animals were the normal control, group II animals served as vehicle control and were administered a single intraperitoneal dose of normal saline, groups III and IV were intraperitoneally injected with a single dose of 30 mg/kg DMN for 48 h, but group IV animals were post-treated orally with 5.71 mg/kg body weight (400 mg/70 kg) ascorbate for seven days, group V animals were pre-treated with same dose of ascorbate orally for seven days before intraperitoneal injection of DMN, while group VI animals were orally administered ascorbate only for seven days. Compared with control, DMN administration resulted in significant decrease ($p < 0.05$) in serum total cholesterol, testosterone (TST), luteinizing hormone (LH), free triiodothyronine (fT_3), and kallikrein III (KLK-3) levels, as well as non-significant increase in serum thyroid stimulating hormone (TSH) level. Pre-treatment with ascorbate significantly increase LH and KLK-3 levels, while post-treatment significantly increase fT_3 level. Also, pre-treatment with ascorbate significantly reduced TSH level, while there was no significant difference in TST level following ascorbate treatments. From our findings and to some extent, ascorbate demonstrates ameliorative effects against DMN-induced hormonal disruption in male wistar rats, and this may be attributed to its antioxidant property.

Keywords: Dimethyl nitrosamine; Ascorbate; Thyroid hormone; Reproductive hormone; Kallikrein-3

1. Introduction

N-nitroso compounds (NOCs) are well established carcinogens present in a vast variety of food stuffs such as smoked fish, dried malt, beer, milk products, meat products, and preserved fruit juices [1–3]. Among various NOCs, dimethyl nitrosamine (DMN) is a well-known carcinogen, mutagen, and hepatotoxin [4,5], present in tobacco smoke, high nitrates containing water, fried meals, cosmetics, pharmaceutical agents, and agricultural chemicals [6].

DMN targets primarily the liver, which contains the necessary enzymes for its metabolic activation. Metabolism in the liver is by a microsomal membrane-bound enzyme, cytochrome P-450 2E1 [7–9]. DMN exerts carcinogenic effects and induces hepatic necrosis through metabolic activation by cytochrome P_{450} 2E1 [10] in experimental animals. The formation of reactive oxygen species (ROS) like H_2O_2, superoxide anion (O_2^-) and hydroxyl radicals ($OH^{\cdot}$) has been demonstrated during the metabolism of nitrosamines resulting in oxidative stress, which may be one of the key factors in the induction of pathological conditions such as hepatocellular

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necrosis, carcinogenicity, neoplastic changes, and tumor formation [11–13].

The most abundant and effective antioxidant in the human body is ascorbic acid [14]. Due to these, the present study checked the effects of treatments with ascorbate on thyroid and reproductive hormones disruption as well as kallikrein-3 level in DMN-induced toxicity in male wistar rats.

2. Materials and methods

2.1. Test substance and kits

DMN (purity ≥ 98%) used in this study were of analytical grade, product of Sigma Chemical Co., Saint Louis, MO, USA. Ascorbate (vitamin C) was purchased from Kunimed Pharmachem Limited, Lagos, Nigeria. Total cholesterol (TCHOL) kit used, is a product of Cypress Diagnostics, Langdorp, Belgium, while TST, LH, fT3, TSH, and KLK III enzyme immunoassay (EIA) test kits were products of Bio-Inteco Diagnostic Limited, Beechwood Road, England.

2.2. Experimental animals and study design

Thirty (30) male wistar albino rats of an average weight of 250 g used for 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. After a long period of acclimatization, the rats were divided randomly into six groups (I–VI) of five animals each, and were administered as presented below:

Group I: animals served as normal control and were served food and water throughout the study.

Group II: animals served as vehicle control and were administered a single intraperitoneal dose of 6 mL/kg normal saline.

Groups III: animals were administered 30 mg/kg single intraperitoneal dose of DMN [15] for forty eight (48) hours only.

Group IV: animals were administered 30 mg/kg single intraperitoneal dose of DMN, followed by oral post-treatment with 5.71 mg/kg (400 mg/70 kg) ascorbate for seven (7) days.

Group V: animals were orally pre-treated with 5.71 mg/kg ascorbate for 7 days, followed by a single intraperitoneal dose of 30 mg/kg DMN for 48 h.

Group VI: animals were orally administered ascorbate for 7 days.

2.3. Sample collections and preparations

At the end of the experimental period, the animals were sacrificed by cervical dislocation. They were handled and used in accordance with the international guide for the care and use of laboratory animals [16]. Blood samples were collected from the abdominal artery into clean plain tubes, and were allowed to stand for 20–30 min; followed by centrifugation at 3000 rpm for 10 min. Serum was separated and aliquoted into clean 1 ml Eppendorf tubes, and stored at −18 °C until when used.

2.4. Determination of total cholesterol concentration

Serum TCHOL was determined according to the methods described in Cypress Diagnostics Kits, Langdorp, Belgium. Briefly, cholesterol esterase hydrolyzed cholesterol esters to release free cholesterol which was oxidized by cholesterol oxidase, and the resulting hydrogen peroxide (H_2O_2) reacted with 4-aminophenazone and phenol to form a red quinonimine dye, whose color intensity is proportional to the cholesterol concentration.

2.5. Estimations of serum levels of TST, LH, fT_3, TSH, and kallikrein-3

These were done as described in Bio-Inteco Diagnostic EIA test kits, based on antibody-antigen reactions. As a result, the developed color intensities which are directly proportional to the concentrations in the test samples were measured spectrophotometrically at 450 nm using BioTek ELx800 Microplate reader (Northstar Scientific Limited).

2.6. Statistical analysis

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.

3. Results

3.1. Effects of ascorbate treatments on serum TCHOL concentration

DMN administration significantly ($p < 0.05$) reduced the concentration of serum TCHOL concentration when compared with control (Fig. 1), while pre- and post-treatment with ascorbate did not have any significant effect ($p > 0.05$) on its levels (Fig. 1).

3.2. Effects of ascorbate treatments on serum LH and TST levels

Compared with control, DMN administration resulted in significant decrease ($p < 0.05$) in LH (Fig. 2) and TST (Fig. 3) levels. Ascorbate pre- and post-treatments resulted into significant ($p < 0.05$) and non-significant increase ($p > 0.05$) in LH levels respectively (Fig. 2), while both forms of treatments did not have any significant ($p > 0.05$) effects on the TST levels (Fig. 3).