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

Oluwatobi T. Somade*, Regina N. Ugbaja, Olubisi E. Adeyi, Damilola M. Ogunberu

Department of Biochemistry, College of Biosciences, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria

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.

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 ($OH^.$) 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

* Corresponding author.
  E-mail addresses: toblerum@yahoo.co.uk, somadeot@funaab.edu.ng (O.T. Somade).

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).
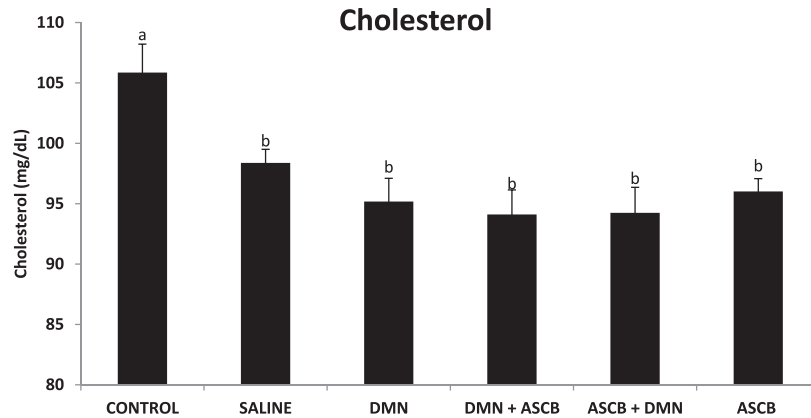
Fig. 1. Effects of pre- and post-ascorbate treatments on total cholesterol levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.

3.3. Effects of ascorbate treatments on serum TSH and fT₃ levels

There was a non-significant increase (p > 0.05) in TSH level (Fig. 4), and a significant (p < 0.05) decrease in fT3 level (Fig. 5), accompanying DMN administration compared with control. Pre-treatment only with ascorbate significantly (p < 0.05) decrease TSH level (Fig. 4), while fT3 level was significantly (p < 0.05) increased by post-treatment with ascorbate (Fig. 5).

3.4. Effects of ascorbate treatments on serum KLK-3 levels

DMN administration significantly (p < 0.05) decreased in KLK-3 level when compared with control (Fig. 6), but only pre-treatment with ascorbate that was able to significantly (p < 0.05) increase the levels back to level comparable with control.

4. Discussion

The present study was carried out to investigate the effects of DMN-induced toxicity on some reproductive, thyroid hormones as well as KLK-3 levels, and the role of pre- and post-ascorbate treatments in rats. One of the key functions of cholesterol in animals is to serve as a pre-cursor to the biosynthesis of steroid hormones [17]. The significant decrease in TCHOL concentrations (Fig. 1) following DMN administration may be due to alteration in the hepatic synthesis of this lipid. DMN-induced toxicity could also have altered other biological molecules that require cholesterol as precursor (e.g. bile acid), and consequently, the availability of the lipid may have been channeled to their synthesis. Some of the compounds that have also been reported to decrease serum or plasma TCHOL are sodium azide [18], and carbon tetrachloride [19].

LH is also referred to as lutrophin [20], and is produced by gonadotropic cells in the anterior pituitary gland. In females, it
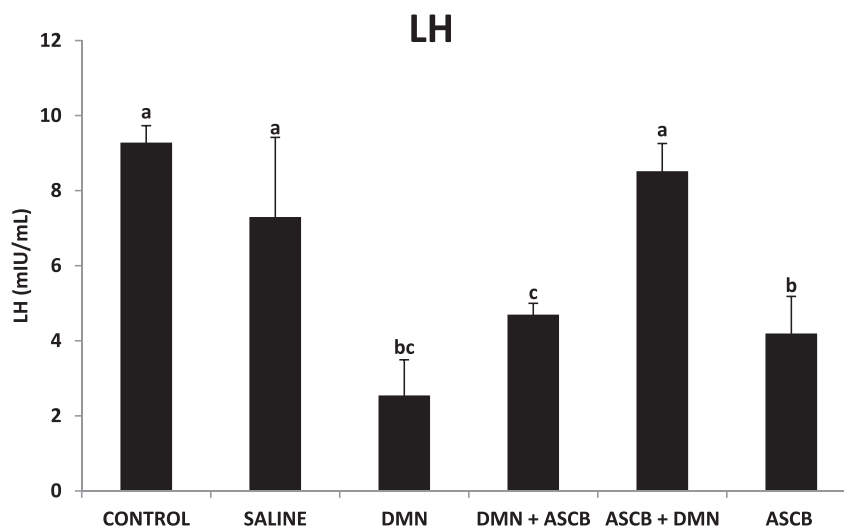

Fig. 2. Effects of pre- and post-ascorbate treatments on LH levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.
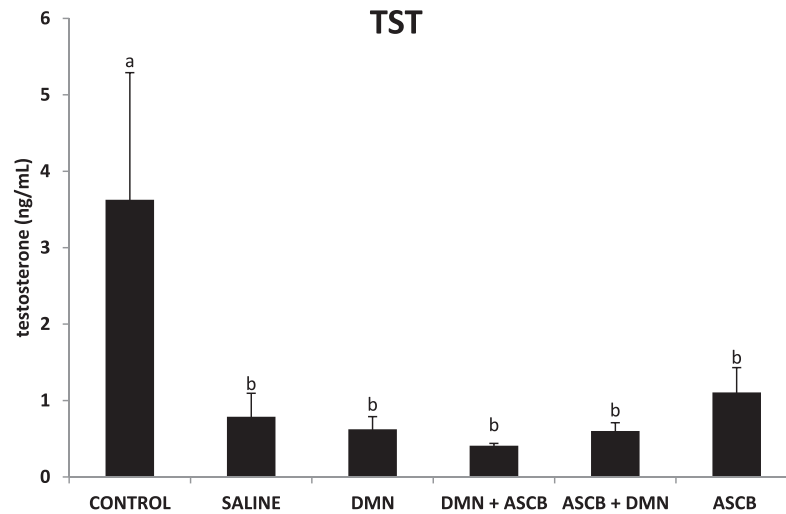
Fig. 3. Effects of pre- and post-ascorbate treatments on TST levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.

triggers ovulation, while in males, it stimulates leydig cells to produce TST [21]. Pituitary gland releases LH, and is controlled by actions of gonadotropin releasing hormone (GnRH). Following low levels of TST, hypothalamus releases GnRH, stimulating the pituitary gland to produce LH [22]. In this present study, DMN-induced toxicity significantly decreased serum LH level (Fig. 2), suggesting an interference of DMN with anterior pituitary, leading to lack of LH secretion. Also, it may be due to decreased secretion of GnRH by the hypothalamus, or failure of the anterior pituitary to respond to GnRH stimulation. Similar findings have been reported in rats following morphine [23−26], and alcohol exposures [27,28]. Streptozotocin-induced diabetic rats have also been reported to have low levels of LH [29]. Amelioration by ascorbate pre-treatment may be attributed to its antioxidant and cyto-protective properties against free radical damage [30,31] that may have protected the hypothalamic−pituitary axis against free radical attack.

TST, a steroid hormone belonging to the androgen class, is found in humans and other vertebrates. 11-ketotestosterone [32] and ecdysone [33] are slightly different forms of TST found in fish and insects respectively. TST plays a major role in the development of male reproductive tissues such as testis and prostate in men. It also promotes secondary sexual characteristics including increased bone and muscle mass, and growth of body hair [34]. Also, TST is important for the prevention of osteoporosis [35], and for health and well-being [36]. Levels of TST are greater in adult male than female adults [37]. The significant decrease in serum TST (Fig. 3) obtained in this study can be attributed to the significant decrease in serum TCHOL recorded, being a precursor to steroid hormone synthesis [38]. LH stimulates leydig cells of the testes to produce TST [21,22]. Therefore, the decreased level of serum TST recorded can be attributed to the low LH levels. Our findings are corroborated by the studies of Bazzano et al. [39] and Ochiogu et al. [40], who reported low serum
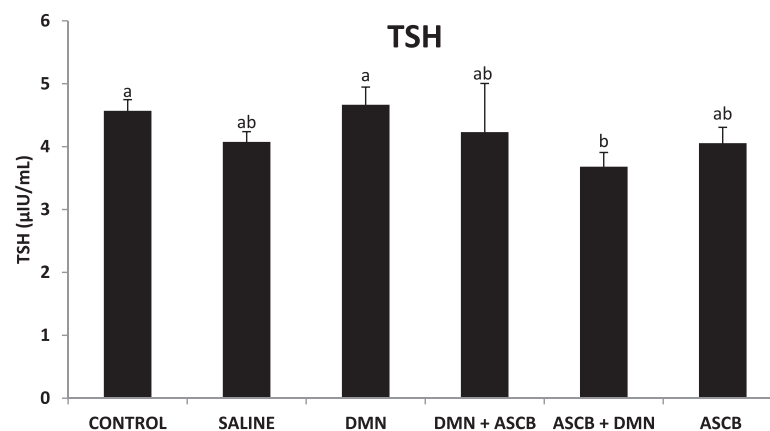

Fig. 4. Effects of pre- and post-ascorbate treatments on TSH levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.
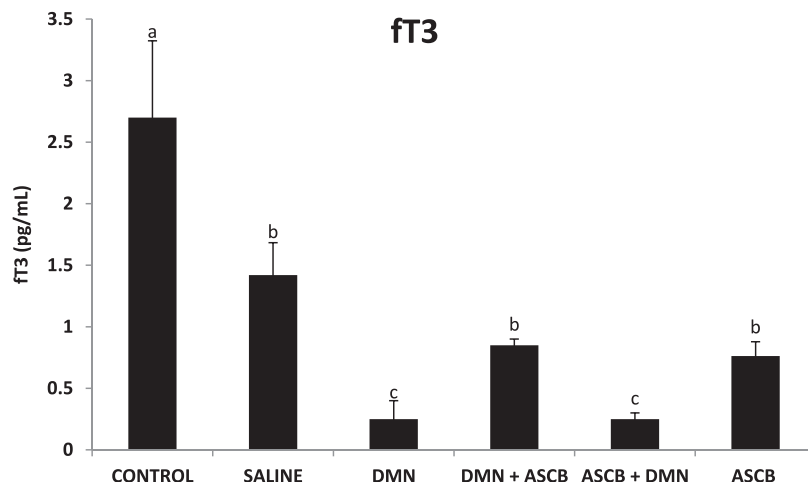
Fig. 5. Effects of pre- and post-ascorbate treatments on fT3 levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.

Fig. 6. Effects of pre- and post-ascorbate treatments on KLK-3 levels in DMN- induced toxicity. Values are expressed as mean ± SEM (n = 5). Bars with different letters are significantly different (p < 0.05), while bars with similar letters are not significant (p > 0.05). DMN = dimethyl nitrosamine; ASCB = ascorbate.
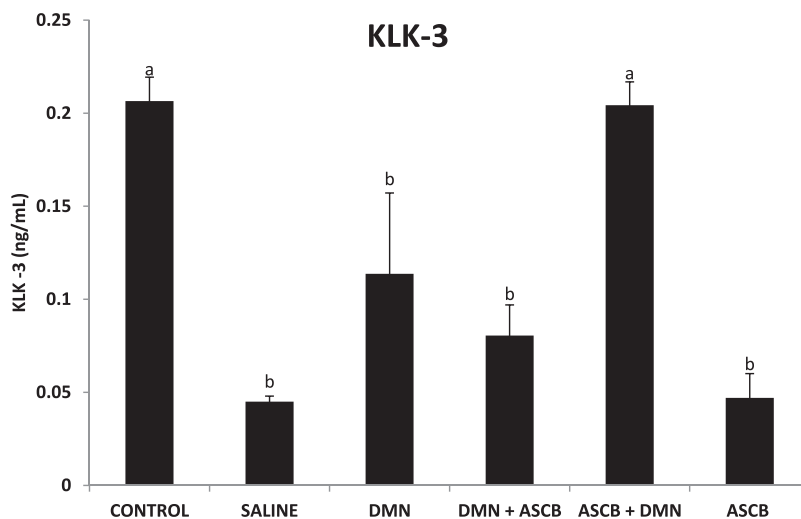
TCHOL, LH and TST following monosodium L-glutamate administration in humans and West African Dwarf goats respectively. Also, Manjunath et al. [29] reported low TST and LH levels in streptozotocin-induced diabetic rats. Free radicals-induced tissue damage has been reported as a mechanism of action of DMN [12,13]. Therefore, ineffectiveness of ascorbate treatment on serum TST levels is suggested to be due to free radical damage on testicular leydig cells [41], rendering them unresponsive to LH stimulation to produce TST.

TSH or thyrotropic hormone is a pituitary and glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, regulating the endocrine function of the thyroid [42]. TSH stimulates thyroid gland to produce thyroxine (T_4), and triiodothyronine (T_3) which is responsible for the metabolism of virtually all tissues in the body [43].

When there is low level of thyroid hormone in the blood, high thyroid-releasing hormone (TRH) is released by the hypothalamus, so high TSH is secreted by the pituitary [44] to produce thyroid hormones [43]. Therefore, from this study, the increase in TSH level (Fig. 4), although not significant following DMN administration may be due to the low level of fT_3 (Fig. 5).

T_3 is a thyroid hormone that affects almost every physiological process in the body. It is circulated in blood almost completely bound to carrier proteins [45], known as thyroxine binding globulin (TBG) [45]. However, only the unbound portion of T3 (fT_3) is known to be the true hormone responsible for biological actions [43]. Only 20% of thyroid hormone produced is T_3, appreciable amount (85%) of the circulating T_3 is therefore formed from T_4 in the liver and pituitary [43]. Also, the significant decrease in fT3 level (Fig. 5) as a result

DMN administration may be attributed to inability of the liver to activate T_4 to T_3, due to hepatic damage. DMN has been reported to have carcinogenic effects and induces hepatic necrosis in experimental animals [10]. As a component of tobacco smoke condensate and certain alcoholic beverages, DMN can induce lung, liver or renal cancers [46,47].

PSA, also known as KLK-3 is a glycoprotein enzyme, secreted by the epithelial cells of the prostate gland. KLK-3 is needed for the ejaculate, where it liquefies semen in the seminal coagulum, enabling sperm to swim freely [48]. KLK-3 is available in serum of men with healthy prostates in small quantities, but is usually elevated in the presence of prostate cancer or other prostate disorders [49]. From this present study, there was a significant reduction in KLK-3 level (Fig. 6). Though DMN is known to be a potent hepatotoxin, mutagen and carcinogen as already reiterated, administration of DMN may not play a role in the etiology of prostate cancer.

5. Conclusion

From our findings, DMN administration led to hormonal disruption and ascorbate treatments demonstrate to some extent, degree of amelioration which may be attributed to its antioxidant [50−52] and cyto-protective properties.

Conflict of interests

The authors declare that they have no conflict of interests.

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