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Short Communication

Combined Effects of Vitamin C and Tomato Extract (Lycopersicon Esculentum) on Carbimazole-induced Alterations in the Testes of Male Albino Rats

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Abstract

Carbimazole administration, in excess, is associated with various pathological conditions, which includes testicular damage. Generation of oxidative stress is one of the plausible mechanisms of carbimazole-induced testicular damage. The aim of the present study was to investigate the effects of carbimazole on testicular function in male albino rats, as well as assess the ameliorative role of the combination of Vitamin C and tomato extracts treatments. Phytochemical analyses of the tomato extracts were performed. A total of 25 male albino rats weighing 200-250 g were randomly divided into five groups (I-V), with five rats in each group. Group I served as normal control and received no treatment. Group II served as the negative control and received only carbimazole (60 mg/kg, oral). Groups III, IV and V served as the treatment groups. They all received carbimazole, and then Vitamin C only (200 mg/kg, oral), tomato extract only (30 mg/kg, oral), and vitamin C plus tomato extract respectively for 3 weeks. Oral administration of carbimazole (60 mg/kg) for 3 weeks resulted in the reduction of serum testosterone level in the negative control rats (group II) from a mean baseline of 0.05±0.00 ng/dl to 0.03±0.00 ng/dl. Histopathological results concomitantly revealed severely eroded seminiferous epithelia and increased interstitial space in this group. However, groups that received daily doses of Vitamin C or tomato extracts alone, or a combination of both, showed attenuated testicular damage, as evidenced by their testosterone levels which differed significantly compared to the negative control ($P<0.05$): 0.05±0.01 ng/dl, 0.09±0.07 ng/dl and 0.04±0.00 ng/dl) for groups III, IV and V respectively. Therefore, a combination of Vitamin C and tomato extract has protective effects against carbimazole-induced testicular damage.

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Introduction

Oxidative stress is defined as a disruption of the equilibrium between oxidant and antioxidant systems. An imbalance between oxidants and antioxidants to produce excess reactive oxygen species (ROS) can cause oxidative damage in vulnerable targets such as unsaturated fatty acids in membranes, thiol groups in proteins, and nucleic acid bases in DNA. Oxidative damage, accumulating during the life cycle, plays a key role in the development of many diseases, testicular disease being one of them. Superoxide dismutase (SOD), malondialdehyde (MDA) and protein disulfide isomerase (PDI) have often been used to demonstrate oxidative damage in tissues (1, 2). 8-hydroxy-2-deoxyguanosine (8OHdG), a compound produced by the oxidation of guanine at the eighth carbon in the nucleus, has been demonstrated immunohistochemically as a specific marker of oxidative stress in rat testes (2)

There are few literatures or researches on the protective effect of antioxidant-rich food and food products on the testis, and there is currently no literature on the effect of tomato extracts against testicular damage induced by toxicant xenobiotics. However, the tomato fruit has been reported to be a rich source of antioxidants such as vitamin C, vitamin E, carotenoids etc (3). Owing to the deleterious and oxidant stress on the testes induced by carbimazole, as extensively reported by Saber *et al.* (1, 4); we evaluated for the comparative effects of two antioxidants (tomato fruit and vitamin C), so as to know if there is synergy in their ability to protect the testes against oxidant injury by carbimazole.

Materials and Methods

Collection and processing of Tomato fruits

Fresh samples of tomato fruit (*Lycoperscon esculentum*) were purchased at Akwata, Ogbete main market in Enugu, Nigeria. The tomato fruits were processed by washing thoroughly in clean water. After washing, they were ground in an electric blender (Saisho, China) at maximum speed for 5 minutes. The extracts obtained were passed through a 52 mm pore size sieve, and were subsequently preserved in the refrigerator at a temperature between 4-6°C for 24 hours.

Phytochemical Analysis of the tomato fruits:

Preliminary phytochemical screening of the tomato fruits was carried out at the Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Procedures outlined by Trease and Evans (5) were employed for the analysis.

Chemical reagents:

ELISA Kits for serum testosterone assay (Elabscience, Texas, USA); Vitamin C (Emzor Pharmaceuticals, Nigeria), to serve as an antioxidant; and carbimazole (Hovid, Malaysia) for induction of testicular damage were utilized for the experiment.

Induction of Testicular Damage

Each experimental rat was administered with high dose carbimazole (60 mg/kg, oral), daily for 21 days.

Experimental animals and maintenance

Twenty-five (25) adult male albino wistar rats, with average weight of (200-250 g), were used in this study. They were obtained from the animal house of the College of Veterinary Medicine, University of Nigeria, Nsukka, Enugu state, Nigeria. The animal experimentation was carried out according to Institutional guidelines describing the use of rats, and approved by the institutional animal research ethical committee (UNTH/CSA. 827/VOL. 17).

Experimental design

The rats were randomly allocated to five (5) groups (I-V) of five (5) animals per group in well ventilated cages. The experimental animals received the following treatments on a daily basis for three weeks together with the stipulated feed and water.

- Group I (Normal Control): No treatment was given to this group.

- Group II (Negative Control) were administered with only carbimazole (60 mg/kg b. wt, oral) for 21 days.
- Group III were administered with carbimazole and the standard drug Vitamin C (200 mg/kg b. wt, oral) for 21 days.
- Group IV were administered with carbimazole and tomato extract (30 mg/kg b. wt, oral) for 21 days.
- Group V received carbimazole, Vitamin C, and tomato extract for 21 days.

Biochemical analysis:

Measurement of serum testosterone level was by ELISA method as described in the ELISA Kits (Elabscience, Texas, USA); and as reported by Engvall *et al.* (6)

Histopathological studies:

The excised testes were fixed in 10% formal saline for 24 hours and further processed using the conventional paraffin wax embedding technique for light microscopic examination. The paraffin-embedded testicular tissues were sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] Staining procedure (7). The histological sections were examined using an Olympus ™ light microscope.

Statistical analysis:

Data were analyzed using SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc. All data were expressed as Mean±SEM. Level Of Significance was determined by the one way analysis of variance (ANOVA) followed by the Tukey's Post-HOC multiple comparison tests. $P<0.05$, $P<0.01$ or $P<0.001$ was considered significant.

Results

Phytochemical results

The result of the preliminary phytochemical analysis of tomato fruit revealed abundant presence of reducing sugar and alkaloids (+++); moderate presence of flavonoids (++); and presence (in trace amount) of carbohydrate and resins (+). However glycosides, saponins, tannins, proteins, oils, acidic compounds, terpenoids and steroids were absent.

Biochemical results

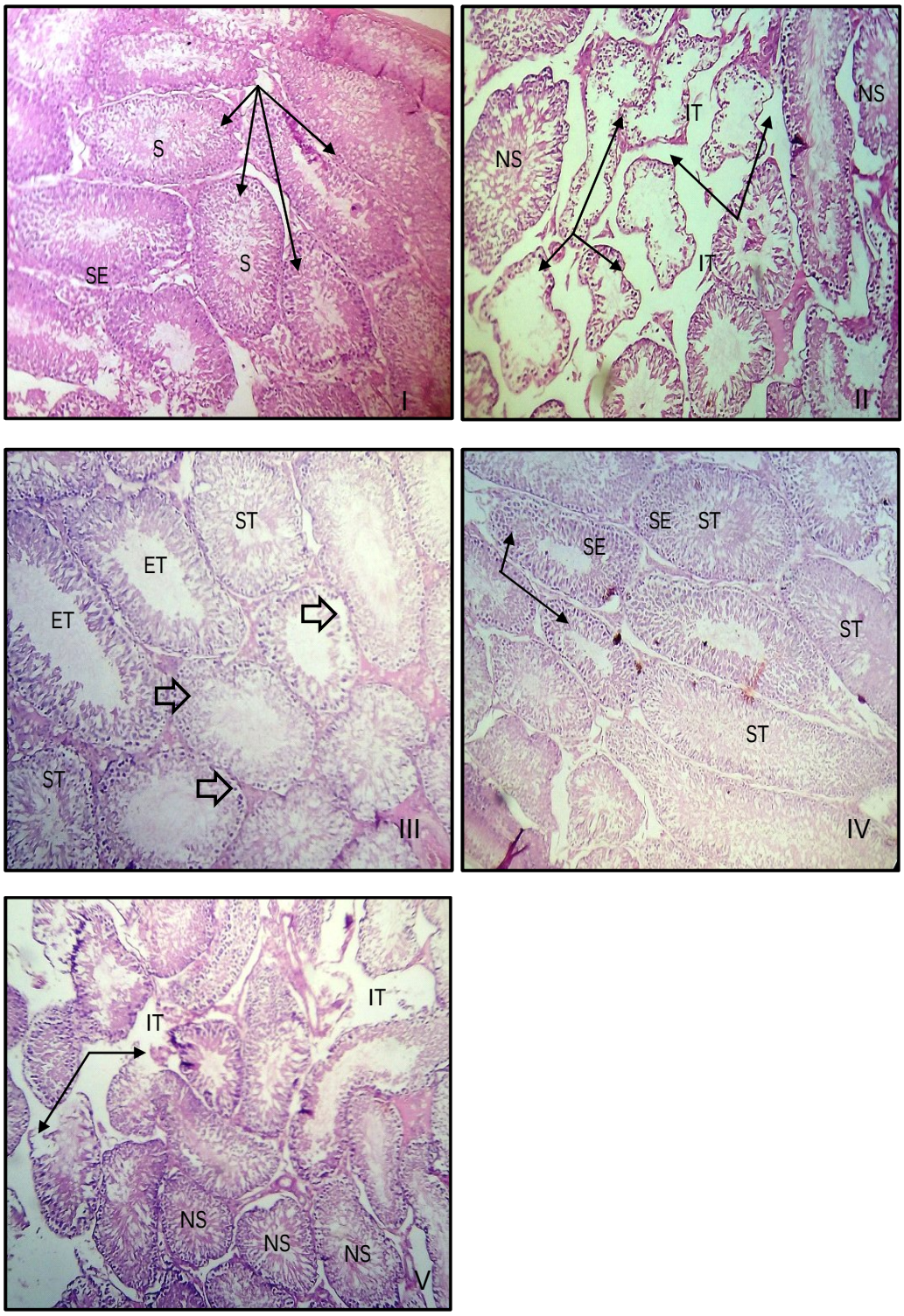
Carbimazole (60 mg/kg, oral) administration for 3 weeks resulted in the reduction of testosterone level in the rats (0.03±0.00 ng/dl) in the negative control rats (group II). Daily administration of Vitamin C alone, tomato extracts alone or the combination of Vitamin C and tomato extract attenuated testicular damage in the rats as evidenced by the serum testosterone levels: 0.05±0.01 ng/dl, 0.09±0.07 ng/dl and 0.04±0.00 ng/dl for groups III, IV and V respectively compared to the negative control which received no treatment ($P<0.05$). There was however no statistically significant difference in the testosterone levels among the treatment groups.

Discussion

Although the blood testes barrier functions as a "gatekeeper" to prohibit harmful substances from altering the normal function of the testes and the positive antioxidant status of the testes, previous studies have shown that some factors (drugs inclusive) are known to disturb these defenses and cause a state of oxidative stress.

Carbimazole, an antithyroid hormone drug, has been scientifically and extensively reported by Saber *et al* (1, 4) to induce testicular damage and oxidative stress; and this is evident in their assay for antioxidant status of the testes, of superoxide dismutase (SOD) and catalase, after oral administration of carbimazole (1.35 mg/kg) for 8 weeks. Furthermore, hypothyroidism has been reported to alter antioxidant defense system in rat (8, 9). Hence, carbimazole has been scientifically proven to cause oxidant stress, at toxic levels, in the system.

Based on this scientific evidence, in this present



Histopathological results

Fig. 1 : Sections of the testes of a representative animal from Group (I-V). In (I), most of the seminiferous tubules appear normal with evenly distributed germinal cells (arrows) in the seminiferous epithelium (SE). Semen and flagella tufts (S) are seen in the lumen of the tubules indicative of complete spermatogenesis. In (II), seminiferous tubules appear shrunken with severely eroded seminiferous epithelium (arrows) and increased interstitial tissue space (IT). A few of the tubules are still normal (NS). In (III), the seminiferous tubules appear normal but their epithelium appears to have scanty germinal cells (block arrow). Some tubules have mature spermatids (seen by the tufts (ST) in their lumen) while others are empty (ET). In (IV), the tubules appear normal with well distributed germinal cells (arrows) in the seminiferous epithelium (SE). Most tubules have mature spermatids (ST). In (V), most of the seminiferous tubules are normal (NS). Others appear to have disrupted basal laminae (arrows). Parts of the interstitial tissue (IT) are also eroded. Stain: Haematoxylin and Eosin, magnification (x100).

study, we administered an overdose of carbimazole (60 mg/kg, oral), over 40 times the dosage Saber *et al.* administered, for 3 weeks. We didn't intend to directly evaluate for the antioxidant properties of the combined effects of Vitamin C and tomato; rather we investigated for their protective ability against the toxic impacts (histoarchitectural damage) by carbimazole on the testicles in albino rats, as evident in our biochemical and histological findings.

The active phytochemicals present in this tomato extract are the flavonoids, and alkaloids. Flavonoids constitute the largest group of naturally occurring phenolics in tomatoes (3). They demonstrate antioxidant activities and have also been shown to possess many health promoting properties. Most of the known alkaloids are related to tissue protection.

Alkaloids are a class of naturally occurring organic nitrogen containing bases which have important physiological effect on both humans and other animals. Lycopene is an antioxidant found abundantly in tomatoes. It has the ability to destroy free radicals, thereby preventing their harmful effects on cells and the immune system (10). Lycopene has also been reported to be very active against prostate cancer (10, 11).

Oral administration of high dose carbimazole (60 mg/kg) daily, to the rats for 3 weeks, caused a decrease in serum testosterone level, and the seminiferous tubules appeared shrunken with severely eroded seminiferous epithelium (Fig. 2II). These results agree with the observations of Saber *et al.* (4), where they demonstrated the cytotoxic effect of carbimazole on testicular tissues.

The carbimazole-induced histological and biochemical alterations in the rats treated with Vitamin C alone (group III) were remarkably improved; the same applied to groups IV and group V which were treated with tomato extracts only and a combination of vitamin C and tomato extracts respectively. Vitamin C and the tomato extracts evidently acted as antioxidants. In these groups, the distribution of germinal cells in the seminiferous tubules appeared better (group III), and normal in (groups IV and V). The improved testicular architecture in these groups could be due to the protective effects of the antioxidants. From the foregoing observations, Vitamin C and tomato extract synergistically protected the testicles of the albino rats in group V.

Conclusion

This study has shown that testicular damage can be induced by the antithyroid drug, carbimazole. However, the combination of Vitamin C and tomato extract has protective effect against carbimazole-induced testicular damage.

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