SOME ADVERSE EFFECTS OF NITRITE ON OXIDATIVE STATUS AND HISTOLOGICAL STRUCTURES OF ADULT MALE WISTAR RATS TESTES

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ABSTRACT

Nitrite is a useful precursor to a variety of organic compounds such as pharmaceuticals, dyes, and pesticides but it is probably best known as food additive to prevent botulism. 20 adult male Wistar rats weighed between 160g - 240g used for this study were obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

The rats were assigned to 4 groups (n=5). The nitrite used was prepared from sodium nitrite. The control group received 1ml of distilled water, while rats of the treated groups received 0.04mg/kgbw, 0.06mg/kgbw and 0.08mg/kgbw nitrite respectively by oral route. The dosing was done for 30 days. Sacrifice of the rats was done by cervical dislocation 4 hours after the last dosing.

There was decreased in Mean body weight of the treated rats. The results also generate oxidative stress in testes. Histological examination shows Seminiferous tubules with focal areas of lost germ cells, hyperplasia of Leydig cells and at 0.06mg/kg dose level,

there was arrest of spermatogenesis while there was no evidence of spermatogenesis at 0.08mg/kg nitrite dose.

These findings suggest that nitrite may change the testicular oxidative status and may play a role in testicular dysfunction that causes infertility. We therefore recommend that excess exogenous nitrite intake should be reduced or avoid.

Key Words: Nitrite, Wistar Rat, Testes, Oxidative Stress, Histological

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INTRODUCTION

Nitrites are a normal part of human diet found in most vegetables **(**Leszczynska, *et al*., 2009; Dennis and Wilson, 2003**)**. Spinach and lettuce can have as high as 2500mg/kg, curly kale (302.0mg/kg) and green cauliflower (61.0mg/kg), to a low of 13mg/kg for asparagus. Nitrite levels in 34 vegetable samples including different varieties of cabbage, lettuce, spinach, parsely and turnips ranged between 1.1 and 57mg/kg **(**Leszczynska, *et al.,* 2009**;** Correia, *et al.,* 2010**)**. Boiling vegetables lower nitrate but not nitrite **(**Leszczynska, *et al*., 2009**)**. Fresh meat contains 0.4 – 0.5mg/kg nitrite and 4 – 7mg/kg of nitrate **(**Dennis and Wilson, 2003**)**. The presence of nitrite in animal tissue is a consequence of metabolism of nitric oxide, an important neurotransmitter **(**Meulemans and Delsenne, 1994**)**. Nitrite can be reduced to nitric oxide or ammonia by many species of bacteria. Under hypoxic conditions, nitrite may release nitric oxide which causes

potent vasolidation. Several mechanisms for nitrite conversion to nitric oxide have been described including enzymatic reduction by xanthine oxidoreductase, nitrite reductase, and nitric oxide synthase (NOS), as well as nonenzymatic acidic disproportionation reactions **(**Ivanov, 2004**).** Nitric oxide, is a free radical **(**Anders, 2010**),** and is an important intermediate in the chemical industry. Nitric oxide is a by-product of combustion of substances in the air, as in automobile engine, fossil fuel power plant, and is produced naturally during the electrical charges of lightning in thunderstorms. In mammals including humans, NO is an important cellular signaling molecule involved in many physiological and pathological processes **(**Hou,1999**)**. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals like nitroglycerine and amyl nitrite were discovered, more than century after their first use in medicine, to be active through the mechanism of being precursors to nitric oxide. Low levels of nitric oxide production are important in protecting organs such as the liver from ischemic damage) Chronic expression of NO is associated with various carcinomas and inflammatory conditions including Type-1 diabetes, multiple sclerosis, arthritis and ulcerative colitis **(**Elizabeth, *et al.*, 1992**)**. Nitric oxide in the air may convert to nitric acid, which has been implicated in acid rain. However, it is an important source of nutrition for plant life in the form of nitrates. Furthermore, both NO and NO_2 participate in ozone layer depletion. Nitric oxide is a small highly diffusible gas and a ubiquitous bioactive molecule **(**Fontijn, *et al.*, 1970**).** Nitric oxide is a known bioproduct in almost all type of organisms, ranging from bacteria to plants, fungi and animal cells (Roszer, 2012). Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle

to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive, yet diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule (Stryer, 1995).

While nitrite prevents the growth of bacteria, it can be toxic in high amounts for animals including humans. Sodium nitrite LDLo is 71mg/kg meaning a 65kg person would likely have to consume at least 4.615g to result in death. Nitric oxide can be created de novo from nitric oxide synthase utilizing arginine or from ingested nitrate or nitrite **(**Southan and Srinivasan. 1998**)**.

Nitrate (NO3), and its chemical cousin Nitrite (NO2), can cause methemoglobinemia, or "blue baby" disease. High nitrate levels can also indicate the presence of other pollutants, such as bacteria or pesticides, as these pollutants may follow the same path as the nitrate into the water supply **(**McCasland, *et al.*, 1985**)**.

Nitrate and nitrite can be an indicator of more serious pollution problems as they are associated with septic waste and agricultural endeavors. Farmers and home owners using nitrate bearing fertilizers often use a variety of pesticides and herbicides which may migrate to ground water supplies **(**McCasland, *et al.*, 1985**)**.

Due to its high solubility in water, nitrate is one of the most common contaminants in rural and suburban areas. In ground water, nitrate originates primarily from fertilizers, septic systems, and manure storage or spreading operations. Nitrate may also occur naturally due to the dissolution of nitrate bearing rock within the aquifer. With surface supplies, contamination can originate from indiscriminate surface water runoff (non-point

sources) or identifiable sources of contamination such as industrial or municipal discharges (point sources) **(**McCasland, *et al.,* 1985**)**.

Nitroglycerin and amyl nitrite serve as vasodilators because they are converted to nitric oxide in the body. The vasodilating antihypertensive drug minoxidil contains an NO moiety and may act as an NO agonist.

Panesar (1999) and Panesar and Chan (2000) tested nitrate and nitrite in vitro in mouse Leydig tumor cells (MLTC-1) and found that both inorganic nitrate and inorganic nitrite inhibited steroidogenesis. Both nitrite and nitrate can endogenously be transformed to nitric oxide (NO) **(**Ellis, *et al.,* 1998**)** and Panesar (1999) and Panesar and Chan (2000) suggested that the inhibitory effects of nitrate and nitrite are through the action of the metabolite nitric oxide (NO), which is an inhibitor of steroid hormone synthesis **(**Cymeryng, *et al.,* 1998; Kostic, *et al.,* 1998; Masuda, *et al.,* 1997; Natarajan, *et al.,* 1997**)**.

MATERIALS AND METHODS

20 male Wistar rats used for this study were obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The rats weighed between 160g - 240g and were housed in cages and fed with rat chow. Drinking water was provided *ad libitum.*

The rats were acclimatized for 2 weeks prior the commencement of the experiment, and were assigned to 4 groups (n=5). The nitrite used was prepared from

sodium nitrite. The control group received 1ml of distilled water, while rats of the 4 treated groups received 0.04mg/kgbw, 0.06mg/kgbw and 0.08mg/kgbw nitrite respectively via oral route. The dosing was done for 30 days, Sacrifice of the rats was by cervical dislocation. Testes of the rats were removed and fixed in Bouin's fluid for histological analysis (Avwioro, 2010).

Oxidative status analysis

Testes were washed separately free of the blood and connective tissues with 1.15 potasium chloride solution. Each testis was weighed using sensitive balance and cut into pieces and homogenized in equal volume of chilled phosphate buffer with 0.1molar concentration and a PH 7.0 using mortal and pestle placed on iced block. The homogenate was poured in a sample bottle for oxidative stress analysis as described by Aebi, 1984; Missra and Fridovich, 1972; Vashney and Kale, 1990.

RESULTS

There was decreased in Mean body weight of the treated rats while the control rats increased in body weight (table 1).

The activities of Lipid peroxidation (MDA) were insignificantly ($P > 0.05$) increased at 0.06mg/kgbw and 0.08mg/kgbw (0.059 ± 0.005 µmol/g protein, 0.059 ± 0.0110 µmol/g protein) higher than the dose of 0.04mg/kgbw (0.050 ± 0.005µmol/g protein) and the control (0.051 ± 0.003µmol/g protein). The Catalase activity value is higher at

0.08mg/kgbw (0.029±0.009 µmol/g protein) when compared with other dose regime and the control. The result is also statistically insignificant (P>0.05).

However, the activities of Super Oxide Dismutase (SOD) was significantly increased at all dose regimes (P< 0.05) with highest increased value at 0.08mg/kgbw (25.90±0.04 µmol/g protein). Thus, the activities of the oxidative markers indicate that the testicular cells were under oxidative challenges (Table 2).

Histological Observations

Histopathologic features in the rats' testes following daily oral doses of nitrite for 30 days were as presented in Figures 1 to 4. Seminiferous tubules with focal areas of lost germ cells, hyperplasia of Leydig cells and incomplete maturation of spermatocytes at 0.06mg/kg dose level, while there was no evidence of spermatogenesis at 0.08mg/kg nitrite dose.

Table 1: Mean body weight before and after nitrite administration; n=5

Group	Initial Mean weight (g)	Final Mean weight (g)
Control	160	172
0.04mg/kgbw	180	178
0.06mg/kgbw	200	197
0.08mg/kgbw	232	215

Table 2: Showing Oxidative Stress Analysis Of Testes (Mean ± S.E.M)

	Control	**0.04mg/kgbw**	**0.06mg/kgbw**	**0.08mg/kgbw**
Lipid peroxidation **(MDA)** µmol/g	0.051±0.003	0.050±0.005	0.059±0.005	0.059±0.011
Catalase **(H_2O_2)** n moles	0.023±0.008	0.016±0.005	0.023±0.011	0.029±0.009
Superoxide dismutase **(SOD) (U/L)**	25.35±0.05	25.60±0.09*****	25.74±0.06*****	25.90±0.04*****

***** P<0.05 otherwise P>0.05 when compared with the control; n=5

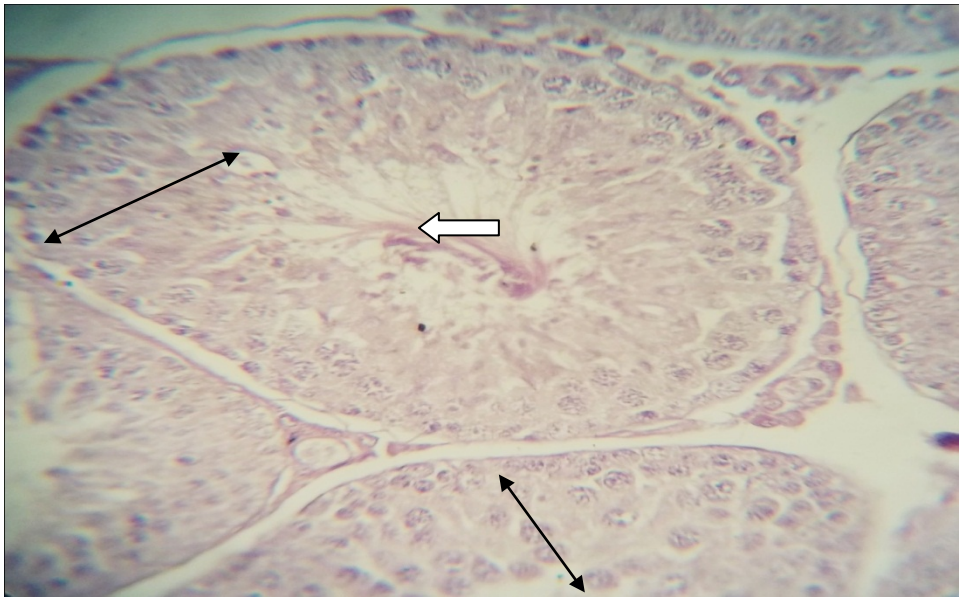

Figure 1: Transverse section of the rat testes (H&E x400) dosed 1ml. deionized water (*control*) daily for 30 days, showing normal seminiferous tubules with complete spermatogenesis (*spanned arrow*).

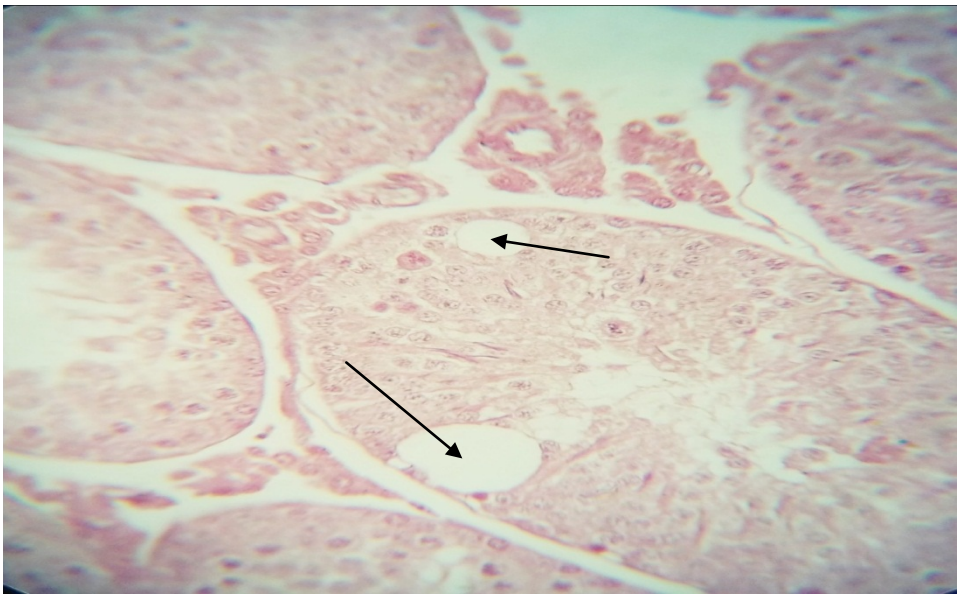

Figure 2: Transverse section of the rat testes (H&E x400) dosed 0.04mg/kgbw nitrite daily for 30 days, showing seminiferous tubules with mild focal area of lost germ cells (*arrow heads*).

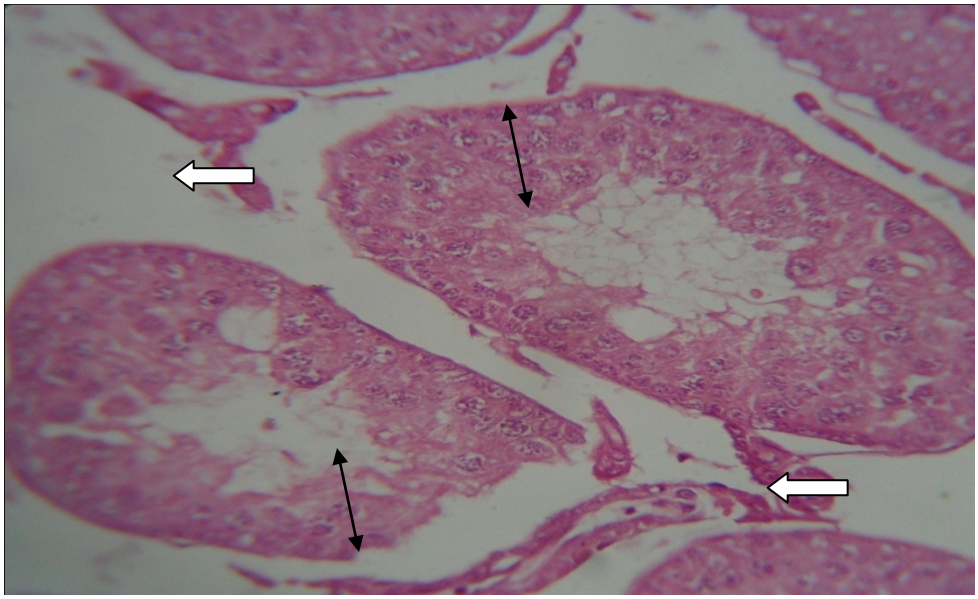

Figure 3: Transverse section of the rat testes (H&E x400) dosed 0.06mg/kgbw nitrite daily for 30 days, showing seminiferous tubules with incomplete maturation of the spermatocytes (*spanned arrows*). The maturation stops at primary spermatocytes, the leydig cells appeared hyperplasia (*white arrows*).

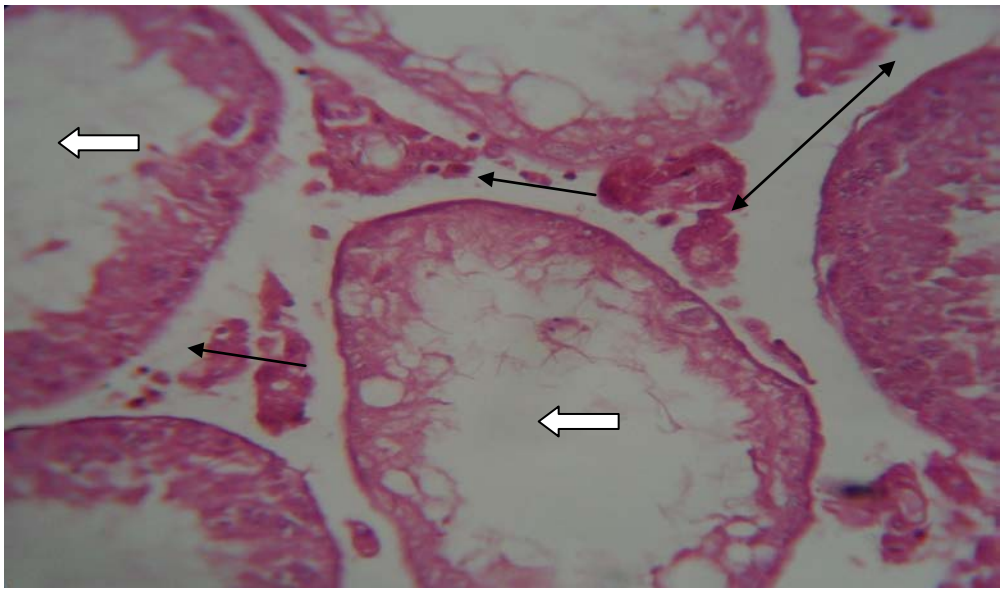

Figure 4: Transverse section of the rat testes (H&E x400) dosed 0.08mg/kgbw nitrite daily for 30 days, showing seminiferous tubules with sloughing of germ cells. leydig cells show hyperplasia (*black arrows*) the wide lumen consist of washed off germ cells (*white arrow*).

DISCUSSION

The reduction in weekly body-weight among the tested rats across the doses (Table 1) coupled with the reported increase in nitrite production with age (Hartman, 1983) and during bacterial infections (Turek, *et. al.*, 1980) respectively, possibly implicates nitrite as an antimetabolite. The slough off germ cells in the seminiferous tubules in the present study was also evident in previous report of increase histologic lesions recorded in seminiferous tubules and epithelial lining of the testes among the tested rats (Akintunde, *et al*., 2010). The hyperplasia interstitium and absence of sertoli cells in the wild seminiferous lumen at 0.08mg/kgbw was similar to the study of Pant and Srivastava (2000) who reported effects on the histopathology of testes in adult male mice after exposure to 900ppm potassium nitrate via drinking water in the study of evaluating of endocrine disrupting effects of nitrate after in utero exposure in rats and of nitrate and nitrite in the H295R and T- screen assay.

Interestingly, Nitric Oxide (NO) has been linked to an increase in lipid peroxidation; malondialdehyde (MDA) in both human varicocele patients (Romeo, *et al.,* 2003) and rats with experimental varicocele (Ozdamar, *et al.,* 2004). This implies a role for peroxinitrites in the oxidative stress of varicocele. and oxidative stress, specifically an increase in NO subsequent to eNOS overexpression, has been linked to germ GCA in a mouse model of cryptorchidism (Ishikawa, *et al.,* 2005). On the contrary, the present study revealed non significant increase MDA and catalase in dose dependent manner.

However, Significant increase superoxide dismutase (SOD) value with increased nitrite dose in the recent study go in similar way with the reported increased NO from a variety

of stresses which decreases testosterone secretion. Also very large increases in NO and formation of peroxinitrites are associated with oxidative stress, which may override the effects of Hypoxia Inducible Factor (HIF)-1α and inhibit testosterone production (Del Punta, *et al*, 1996; Kostic, *et al.*, 1998; ; Zhou, *et al.*, 2003).

In the present study the reactive oxygen specie triggered by nitrite would generate oxidative stress and histological damages. Therefore, we recommend that excess exogenous nitrite intake should be reduced or avoid.

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