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Oxidative damage on the testes of adult rats by sodium metabisulfite (MBS)

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ABSTRACT

The effect of sodium metabisulfite (MBS, $Na_2S_2O_5$) on the level of malondialdehyde (MDA), testes weight and the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) as well as the level of glutathione (GSH) were investigated in the testes of Wistar albino rats. The study was performed to elucidate the effect of MBS (520 mg/kg/day) ingestion on lipid peroxidation and the antioxidant enzymes activities. The results showed that MDA level as well as the activity of SOD was significantly increased in MBS-treated rats whereas the weight of the testes as well as the level of GSH was not significantly affected. However, there was significant reduction in the activity of CAT in the MBS-treated rats compared to the control. The presented data showed that ingestion of MBS has adverse effect on testes as it induced oxidative damage, reflected by increase lipid peroxidation and impairment in the activities of antioxidant enzymes.
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Keywords: Sodium metabisulfite, testes, lipid peroxidation, glutathione, catalase, superoxide dismutase

INTRODUCTION

Sulfite and related chemical such as sulfite salt and sulfur dioxide has been used as preservatives in food and drugs. Considerable amount of sulfites is generated *in vivo* from the catabolism of sulfur-containing amino acids such as cysteine and methionine (Ozturk et al., 2010; Elmas et al., 2005). Five sulfite salts including sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3) are commonly used as antioxidants in food preparations (Elmas et al., 2005). In addition to this, studies have postulated that under certain conditions, sodium metabisulfite may act through cell disruption and compromise membrane permeability and lipid peroxidation (Derin et al., 2006).

Sulfite can cause allergic reactions in humans, produces a variety of toxic effects in animals and may act as co-carcinogen (MacPherson, 2001; Gordon et al., 2004; Ercan et al., 2010). In humans, deficiency of sulfite oxidase, an enzyme which catalyses the oxidative detoxification of sulfite, leads to

mental retardation, neurological symptoms such as spastic quadriplegia and early death (Niknahad and O'Brien, 2008).

Testis is the organ that produces sperm, the male reproductive cell, and androgens, the male hormones. Testis is sensitive to a variety of stressors and exposure to agents that induce germ cell apoptosis (O'Bryan et al., 2000). This organ has fairly high concentrations of antioxidant and the defense against the oxidative stress plays critical roles in the maintenance of spermatogenesis and prevention of testicular atrophy. The present study was aimed at determining the effect of sodium metabisulfite ingestion on antioxidants and lipid peroxidation in the testes of adult rats.

MATERIALS AND METHODS
Animals and chemicals

All reagents were of analytical grade and obtained from sigma chemical Co., USA and BDH chemical Ltd; Poole, England. All experimental protocols conducted on rats were performed in accordance with international standard on animal ethics. Male albino Wistar rats weighing 150-200 g used for the experiment were obtained from animal house of the University of Ibadan, Ibadan, Nigeria. They were allowed to acclimatize for a period of two weeks and maintained at 12 h light-dark cycles at room temperature. At the end of two weeks, rats were randomly divided into two groups: A and B with seven (7) rats per group and kept in different wire cages. The two groups fed, *ad libitum,* on growers mash and water and the weight of rats were taken weekly. At the beginning of second week of the commencement of the experiment, animals in group B were given 520 mg/Kg/day MBS ($Na_2S_2O_5$) by intragastric intubation for 21 days (Hui et al., 1989). At the end of 21-day treatment period, rats were anaesthetized with diethyl ether and

sacrificed. The testes were removed, weighed and kept frozen in the freezer at -4 °C.

Preparation of testes homogenate

Two sets of testes homogenates were prepared. 0.5 g of the testis of individual rat was homogenized in 5 ml 0.01 M phosphate buffer, pH 7.4 at 4 °C and kept frozen in the freezer to be used for Catalase, Glutathione and Lipid Peroxidation analyses while another 0.5 g of the testis was homogenized in 1 ml of isolation buffer (250 mM Sucrose, 5 mM Tris, 1 mM Mercaptoethanol and 0.5 mM Phenylsulphonyl-flouride (PMSF) pH 7.4 at 4°C), also kept frozen in the freezer at -4 °C and used for Protein determination and Superoxide dismutase analyses. Each set of the homogenates was centrifuged (4,000 g, 5 mins, 4 °C) and supernatants were stored in the freezer at -4 °C.

Biochemical analyses
Measurement of lipid peroxidation

Levels of MDA, a product of lipid peroxidation, were measured using the thiobarbituric acid (TBA) according to the method of Vashney and Kale (1990). The method is based on formation of pink colored product (when Malondialdehyde is treated with 2- thiobarbituric acid) which has a maximum absorbance at 531.87 nm.

Assay of glutathione

Assay for glutathione (GSH) was done by the method of Beutler et al. (1963). This method is based on the development of a stable yellow color when 2-nitrobenzoic acids is added to sulfhydryl compounds. The absorbance was measured at 412 nm and the value of GSH is proportional to the absorbance at this wavelength.

Catalase assay

The catalase activity of each testis sample was determined by the method of

Sinha (1972) but with a slight modification. 0.1 ml of each testis was mixed with 4.9 ml of distilled water. 1 ml of the mixture was added to H_2O_2 –phosphate buffer mixture. The principle is based on the formation of chromic acetate when hydrogen peroxide (H_2O_2) reacts with dichromate-glacial acetic acid mixture at 100 ^{O}C. The decomposition of H_2O_2 when acted upon by catalase and reduction of the orange color to a green coloration is measured spectrophotometrically at 570 nm.

Superoxide dismutase (SOD) assay

Total SOD activity was determined by the method of Del-Maestro et al. (1983). This assay reaction is initiated by adding pyrogallol solution and the change in optical density at 420 nm is recorded. The principle was based on the ability of SOD to scavenge superoxide anion radical (O_2^-) which, by shortening reaction chains, decreases the overall rate of pyrogallol autoxidation.

Protein determination

In all measurements of antioxidant system and oxidation products, the protein concentrations were evaluated according to Lowry et al. (1951) with bovine serum albumin as standard. The assay measured at

750 nm was based on the use of Folin-Ciocalteau reagent (a solution of phosphomolybdic-tungstic complex) which is reduced by tyrosine residues in protein at alkaline pH to give blue color.

Statistical analysis

All values were expressed as mean ± standard error of mean (SEM), and the data were analyzed using independent t test for significant differences between the two groups. A level of p<0.05 was accepted as statistically significant (SPSS software).

RESULTS

As shown in the table, there was no significant difference (P>0.05) in the weight of MBS-treated rats when compared with the control. However, result showed that there was a significant increase (P<0.05) in lipid peroxidation and SOD activity of MBS-treated rats and significant decrease (P<0.05) in catalase activity when compared to appropriate controls. In addition, no significant difference was observed in GSH level of MBS-treated rats when compared with the control.

Table: Effects of sodium metabisulfite (MBS) administration on lipid peroxidation and antioxidant levels in the testes of adult rats.

Treatments	Weight (g)	Lipid peroxidation (MDA) x 10^4	Catalase activity (Units g $^{-1}$ tissue) x 10^4	Superoxide dismutase (ng SOD m g^{-1} protein) x 10^3	GSH (mg g^{-1} tissue)
Control n=7	2.34 ± 0.10	26.01 ± 0.01	1.83 ± 0.40	3.24 ± 0.51	213.00 ± 37.77
MBS-Treated n=7	2.18 ± 0.15	58.09 ± 1.80 *	1.00 ± 0.02 *	5.82 ± 0.97 *	252.53 ± 21.73

*P<0.05 Values were expressed as Mean ± S.E.M; Sodium Metabisulfite (520mg/Kg/day MBS ($Na_2S_2O_5$)) was given by intragastric intubation for 21 days. All animals in both groups were maintained on their diet and water throughout the experiment.

DISCUSSION

Free radical-induced oxidative damage has been implicated in the pathogenesis of a number of injuries and disease states (Adebayo and Adenuga 2007; Adenuga et al., 2009). The ability of sulfite to cause peroxidation of lipids has been documented in various fatty acid and lipid emulsions (Zaloga and Marik, 2003). Lipid peroxidation is known to have deleterious effects on structure and functions of cell membrane. Considering that unsaturated fatty acids that undergo peroxidation are important constituents of biological membranes, structural and functional deterioration of biological membrane may follow (Meng, 2003). The level of malondialdehyde (MDA) has been shown to be an indicator of endogenous lipid peroxidation (Meng and Bai, 2004). Our result showed that MBS (520 mg/kg/day) given by intragastric intubation for 21 days, significantly increased the level of MDA in the testes of adult rats. This result is consistent with other previous reports (Aydin et al., 2005; Derin et al., 2008; Ercan et al., 2010). The mechanisms of sulfite-induced lipid peroxidation have been shown to involve SO_3^{2-} oxidation into a sulfite radical ($SO_3^{\cdot-}$). The sulfite radical can subsequently react with molecular oxygen to form sulfite peroxy radical ($SO_3OO^{\cdot}$) and a sulfate radical ($SO_4^{\cdot-}$), which in turn reacts with lipids (Elmas et al., 2005; Ercan et al., 2010). Also, the one-electron oxidation of bisulphate produces the sulfur trioxide radical anion, which reacts rapidly with molecular oxygen to form peroxyl radical. The free radicals generated can damage nucleic acids and induce mutation (Meng, 2003; Meng and Bai, 2004).

Our result on the weight of testes of MBS-treated rats compared with control was not statistically significant. However, there was reduction in the testes of MBS-treated rats compared with the control. The reduction at the testicular weight could be attributed to the reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells (Maneesh et al., 2005). In addition, generation of highly reactive oxygen species (ROS) is another important factor in the damage of this tissue (Adebayo et al., 2011). Oxidative stress in the testes has been reported to be one of the major factors inducing germ cell apoptosis (Maneesh et al., 2005). Our result on lipid peroxidation supports this fact.

In normal condition, cell possesses enzymatic and non enzymatic defenses to cope with free radicals (Adenuga et al., 2008). It has been suggested that many of the clinical and pathological manifestations result from an imbalance between free radical defense system and free radical production. The increase in free radical production has increased the potential for free radical-mediated tissue damage (Ercan et al., 2010).

Superoxide dismutase is a metalloprotein that dismutases $O_2^{\cdot-}$ to yield hydrogen peroxide (H_2O_2) and oxygen. At pH 7.4, the rate constant for this enzyme-catalyzed reaction is $\sim 2 \times 10^9\, M^{-1}S^{-1}$ which is approximately 10^4 times faster than the spontaneous dismutation of $O_2^{\cdot-}$ (Elmas et al., 2005). The result of the present study showed that there was significant increase in the activity of SOD of MBS-treated rats when compared to the control. The result of this study is at variance with the report of Meng and Bai (2004) who reported that exposure to sulfur dioxide decreased the activity of SOD as well as increased the catalase activity on the testicle of mice. The variations in the activities of these antioxidants might be due to the sources of sulfites, the species of animal used and the routes of administration. The increase activity of SOD in the dismutation of superoxide radicals is expected to result in

increase hydrogen peroxide generation. SOD is considered as the first line of defense against oxygen toxicity (Meng and Zhan, 2001; Meng and Zhang, 2003). SOD spontaneously dismutases O_2^- to form (oxygen) O_2 and H_2O_2. Thus, SOD protects testes against spontaneous O_2 toxicity and lipid peroxidation. The H_2O_2 formed from SOD and following divalent reduction of oxygen is removed by either glutathione peroxidase (GPx) which catalyses the reduction of H_2O_2 by reduced glutathione (GSH) (Elmas et al., 2005) or catalase, another primary scavengers of hydrogen peroxide (Laggner et al., 2005), which cannot be saturated by H_2O_2 at any concentration. SOD and CAT also remove (O_2^-) generated by NADPH oxidase in neutrophilis and may play an important role in decreasing lipid peroxidation (Maneesh et al., 2005). Our result showing reduction in the activity of catalase suggests that there was repression of CAT activity and that CAT was not able to remove the hydrogen peroxide released by the action of SOD leading to intracellular increase of hydrogen peroxide.

GSH is a major non protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense processes. Conditions that perturb intracellular levels of glutathione have been shown to result in significant alteration in cellular metabolism. The tissue glutathione concentration reflects its potential for detoxification and it is critical in preserving the proper cellular redox balance and for its role as a cellular protectant (Mari et al., 2001). It is a cofactor for GPx, which catalyses the reduction of hydrogen peroxide to water, thereby limiting the formation of hydroxyl radical, the most toxic of the oxygen-based radicals (Meng and Bai, 2004). As shown in the table, no significant difference was observed in the GSH level in the testes of MBS-treated rats compared to the control. It is well known that the concentration of glutathione in the tissue is a reflection of its potential for detoxification. Thus, it seems that GSH did not play a significant role in scavenging free radicals generated during MBS-induced lipid peroxidation. From the present study, it can be concluded that ingestion of sodium metabisulfite (MBS, $Na_2S_2O_5$) has an adverse effect on testes as it induced oxidative damage, reflected by increase lipid peroxidation which was accompanied by changes in antioxidant status in this organ. It is recommended that further work be done to understand the effect of MBS on sperm and male hormones production.

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