

Original Article**Effect of Sodium Metabisulfite on Rat Ovary and Lipid Peroxidation***Nahid Rezaee¹, Zahra Nematollahi¹, Shahnaz Shekarfroush^{*1}, Ebrahim Hoseini²**Received: 15.06.2015**Accepted: 27.07.2015***ABSTRACT**

Background: Many health problems are related to lifestyle and dietary factors. Since ancient times, food additives such as sulfites have been used to preserve foods. Diverse effects of sulfites on multiple organs have been reported but its effect on female reproductive organ has not been fully elucidated. The aim of this study was to investigate the effects of sodium metabisulfite (SMB) on ovarian tissue in adult rats.

Methods: Four groups of female rats (n=32) were used. The experimental rats received 10, 100 and 260 mg/kg SMB for 28 days (S10, S100 and S260 groups, respectively). The control rats received distilled water for the same period. The ovarian volume, weight and the number of different types of follicles were estimated by stereological methods. Lipid peroxidation is assessed indirectly by the measurement of malondialdehyde (MDA), using the thiobarbituric acid (TBA) method.

Results: The results showed a significant decrease in the ovarian volume, the number of primordial, primary, secondary, graafian follicles and corpus luteum in the SMB-treated animals compared with the control group ($P < 0.05$). In comparison to the control group, the number of atretic follicles increased in the SMB-treated rats. MDA was significantly increased in S260 group compared to the control group.

Conclusion: The present data confirm sulfite-induced structural changes in the ovary. Increased level of MDA because of SMB ingestion suggests that free radicals may have a critical role in these changes.

Keywords: Lipid Peroxidation, Ovary, Sodium Metabisulfite.

IJT 2016 (2): 23-28**INTRODUCTION**

Sulfite agents are widely used as preservative and antioxidant additives in food and pharmaceutical industries. The amounts of sulfite used in foods and beverages vary greatly in different countries. Acceptable Daily Intake (ADI) for sulphur dioxide and sulphites has been determined as 0-0.7 mg/kg. However, the daily intake of sulfite is more than this value. It is possible to consume 180–200 mg/kg sulfite from foods and beverages in a single day or meal [1]. Many types of biological and toxicological effects of sulphites in multiple organs of mammals have been reported [2, 3]. Sulfite induced toxicity is mediated through free radical formation and associated with oxidation of lipids, proteins and nucleic acids [1].

Overproduction of free radicals caused by several chemical and physical agents creates an

environment unsuitable for normal female physiological reactions including many aspects of reproduction [4]. Malondealdehyde (MDA) is one of the major products of peroxidized polyunsaturated fatty acids and increase of MDA content is a significant indicator of lipid peroxidation [5]. Germ cells and follicles are sensitive to toxicants, which induce oxidative stress. Oxidative stress may be a cause of apoptosis in antral follicles and poor oocyte quality [6].

The quantitative parameters of the ovary including the volume of the ovary, the number of the preantral, antral, and atretic follicles can be detectable using stereological methods [7]. The study of these parameters can provide important information about the function of the ovary. The differential follicle counts provide a sensitive means of estimating the extent of chemically induced ovarian toxicity [8].

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The present research was conducted in order to quantify the histological parameter and lipid peroxidation of rat ovaries after SMB treatment.

MATERIALS AND METHODS

Animals and Tissue Preparation

Thirty-two female Wistar rats weighing 220-250 g obtained from animal house of Shiraz University of Medical Sciences, Iran, were randomly assigned to either control (n = 8) or experimental (n = 24) groups. The animals were kept under constant conditions of light (12 h/light/dark/cycle) and temperature (21–24 °C). All the rats had *ad libitum* access to food and water.

All investigations were conducted in accordance with the ethical principles of the use of laboratory animals adopted by Islamic Azad University. The study was approved by Ethics Committee of the university.

The rats in the control group were gavaged with distilled water and the experimental rats were gavaged with SMB (10, 100 and 260 mg/kg) for 28 d. After that, daily vaginal smears were taken to determine ovarian cyclicity and all ovaries were collected in the estrus stage. The rats were euthanized by diethyl ether 24 h after the last administration. The right ovaries were briefly washed in ice-cold 0.9% saline (w/v), frozen in liquid nitrogen, weighed and stored at -70°C until the subsequent protein and MDA assays. The left ovaries were removed and

weighed. Then, the ovaries were fixed in 4% formaldehyde buffer. For isotropic uniform random sections, each ovary was embedded in a spherical module filled with paraffin, randomly rotated, and serially sectioned (sections of 26 μm thickness for number and 5 μm for volume), using a Leitz Rotary Microtome. Eight to twelve sections from each ovary were sampled through systematic random sampling and stained by Hematoxylin and Eosin [7].

Estimation of the Volume of the Ovary

The Cavalieri method was used to estimate the total ovarian volume (Figure 1), using a stereomicroscope connected to a computer at 40x magnifications. Ten sections were sampled and the volume was obtained by point counting method and the following formula [9]:

$$V = \Sigma p \times (a/p) \times t$$

Where “ Σp ” is the total number of points superimposed on the sections; “ a/p ” is the area per point, and “ t ” is the distance between the sampled sections. Additionally, “ a/p ” is calculated by the following formula:

$$(a/p) = (\Delta x \times \Delta y) / m^2$$

Where “ Δx ” and “ Δy ” are the distance between the two adjacent points on the grid in the x-axis or the y-axis, respectively. Moreover, “ m ” is the final linear magnification of the microscopic images [10].

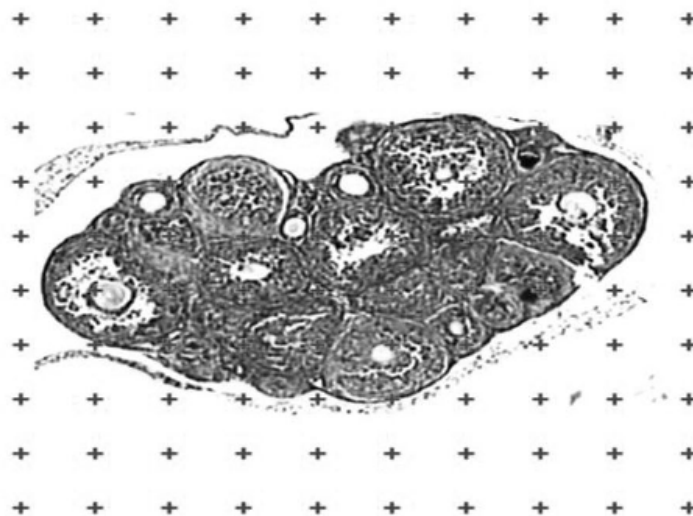


Figure 1. Estimation of the ovary volume using the Cavalieri principle. A grid of points was laid out over the ovarian sections, and the total number of points hitting the sections was counted.

The Number of Different Follicles

The stage of follicles was determined based on the Mayer et al. classification [11]. The number of follicles was determined using an optical disector method with Nikon E200 microscope (Nikon, Japan) with 100x magnification and the microcator (MT12, Heidenhain, Germany) connected to a computer. The unbiased counting frame was superimposed on the monitor images of the 26 μm sections of the ovary and the oocyte nuclei were counted if they lied within the frame and did not touch the exclusion boundaries. On average, 80-100 microscopic fields were selected in each ovary via a systematic sample.

The number density [12] of different types of follicles was estimated using the following formula:

$$N_v = \Sigma Q / \Sigma p.a/f.h$$

where “ ΣQ ” is the total number of the counted follicles, “ h ” is the tissue thickness (10 μm) considered for counting, “ a/f ” is the frame area in the true tissue scale and “ Σp ” is the total number of the points superimposed on the selected fields. The result of the equation was then multiplied by the total volume of the ovary to obtain the total number of follicles [10, 12].

Lipid Peroxide

Lipid peroxidation is assessed indirectly by the measurement of malondialdehyde (MDA), as a marker of cell membrane injury. The level of MDA was determined in the tissue samples homogenized in a ratio of 1/10 in 1.15% (w/v) cold KCl solution, using thiobarbituric acid (TBA) method. MDA reacts with TBA and produces a pink colored complex, which has the maximum absorbance at 532 nm. The results

were expressed as nmol/mg protein. Protein concentration of the tissues was measured by Bradford method [13].

Statistical Analysis

The results were analyzed by one-way ANOVA, Tukey test, using SPSS Version16.0 (Chicago, IL, USA) software and the means were considered significantly different at $P < 0.05$. The data were presented as mean \pm SEM.

RESULTS

The Weight and Volume of Ovary

In comparison to the control group, the weight and volume of the ovary decreased in the SMB treated rats. However, the weight of ovary in the SMB groups was not significantly different from the controls. The volume of the ovary in the S100 and S260 groups showed a significant reduction (34% lower) compared to the control group ($P < 0.01$) (Table 1).

The Number of Different Follicles

A significant decrease was observed in the mean number of the primordial, primary, secondary, grafian follicles, and corpus luteum in the SMB groups in comparison with the controls. The mean number of atretic follicles increased significantly in the S100 and S260 groups compared to the control group (Table 2).

Effect of Sodium Metabisulfite on Ovarian Lipid Peroxidation

Ovarian MDA level increased in the SMB treated rats compared to the control group (47.6 ± 9.3 nmol/mg protein); it was significant in the S260 group (147.2 ± 30.7 nmol/mg protein, $P = 0.01$) (Figure 2).

Table 1. Comparison of the mean of weight and volume of the ovary in the control and sodium metabisulfite (SMB) treated groups 28 days after treatment.

Groups (N = 5)	Weight of ovary (mg)	Volume of ovary (mm ³)
Control	30.4 \pm 3	16.4 \pm 1.2
S10	28 \pm 1.2	14.8 \pm 1.4
S100	24 \pm 1.5	9.6 \pm 0.8**
S260	22 \pm 1.9	10.2 \pm 0.4**

Values are mean \pm SEM. ** $P < 0.01$ significant difference vs. the control group.

S10, administration of 10 mg/kg/day SMB; S100, administration of 100 mg/kg/day SMB; S260, administration of 260 mg/kg/day SMB.

Table 2. The total number of different follicles in the ovary of the control and SMB treated rats.

Groups	Follicle Types					
	primordial	Primary	Secondary	Graafian	Corpus Luteum	Atretic
Control	1658 ± 210	1608 ± 168	627 ± 114	130 ± 44	1194 ± 48	503 ± 48
S10	1562 ± 172	1487 ± 95	549 ± 58	120 ± 40	1103 ± 30	526 ± 60
S100	740 ± 244*	472 ± 141***	178 ± 70**	21 ± 8	780 ± 112**	2088 ± 463**
S260	553 ± 140**	223 ± 51***	116 ± 52**	7 ± 5*	641 ± 145*	2713 ± 282***

Values are mean ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significant difference vs. the control group. S10, administration of 10 mg/kg/day. SMB; S100, administration of 100 mg/kg/day. SMB; S260, administration of 260 mg/kg/day SMB.

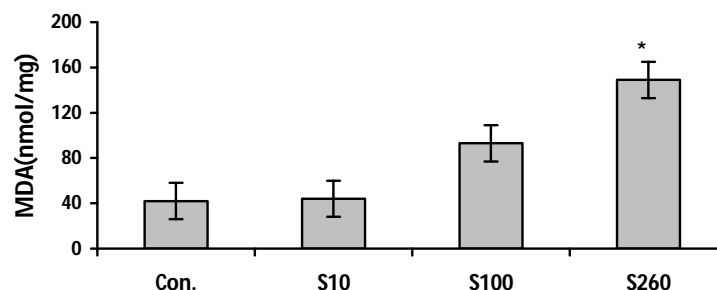


Figure 2. Ovarian malondialdehyde (MDA) levels on day 28 of experiment. Data are presented mean ± SEM. * $P < 0.05$ compared with control.

DISCUSSION

The present study evaluated the ovarian structure in the rats treated with sodium metabisulfite (SMB). SMB induced structural changes in the rats' ovaries. The main structure changes included a reduced volume of the ovary as well as a decrease in the number of primordial, primary, secondary, graafian follicles, corpus luteum and an increase in the number of atretic follicles. In addition, SMB resulted in increased lipid peroxidation in the ovarian tissue of the rats when given doses of 260 mg/kg/d.

In reproductive medicine, the ovarian volume can be used for estimation of the remaining follicle pool for women. Therefore, it has a role in assessment of the ovarian reserve, which is an important factor in the fertility potential. A reduced volume is a predictor of poor outcome for assisted conception [14].

The growth and development of the ovarian follicles is under the tight control of endocrine, paracrine, and autocrine factors [11, 15]. Anything that has a destructive impact on these factors may impair the development of the ovarian follicles. The most sensitive quantitative indicator of ovarian toxicity is the differentiation

of the follicle counts [16]. Chemicals, which extensively destroy the primordial follicles, can lead to irreversible infertility and premature ovarian failure [17]. The survival of the primordial follicles is important as a reserve to provide the oocytes throughout the reproductive life [18]. The present results indicate that following repeated exposure to SMB, the differential follicle counts were decreased.

Due to the excessive use of food additives in the last few decades, it is estimated that each person consumes these compounds more than the acceptable daily intake. Exposure to sulfites can occur through foods, the use of cosmetics including hair colors, bleaches, creams, and perfumes, and the use of medicines including eye drops, topical medications, and parenteral medications such as adrenaline, phenylephrine, corticosteroids and local anaesthetics [19].

Sulphur dioxide and its salts are known to present allergic reactions in humans, produce some toxic effects in animals and may act as co-carcinogen [2, 19, 20]. Sulfites also affect male reproduction. Sulfur dioxide treatment resulted in higher LDH activities and lower protein levels in the testes [21]. The ingestion of sodium metabisulfite (520 mg/kg/d) induced oxidative

damage, reflected by increased lipid peroxidation and impairment in the activities of antioxidant enzymes in the testis of rats, and these changes may be one of the pathways that cause low sperm motility [2]. In the culture medium, in the cells treated with sodium bisulphite, a reduction of protein content and colony-forming ability in both number and colony size was detected [22].

Lipid peroxidation is involved in several disease states including diabetes and cardiovascular diseases as well as the aging process [23]. Oxidative stress affects the reproductive lifespan of a woman by mechanisms such as lipid peroxidation, inhibition of protein synthesis and DNA damage. There is a delicate balance between ROS and antioxidant enzymes in the ovarian tissues; however, pathologically high concentrations of ROS may promote the cell death [24]. Therefore, the observed increase in MDA as the marker of lipid peroxidation in the present study may be one of the mechanisms that impair the intracellular milieu, resulting in diseased cells or endangered cell survival. Lipid peroxidation following sulfite treatment is in agreement with the finding of Ozturk et al. [1]. Sulfite-induced lipid peroxidation has been attributed to sulfite oxidation into a sulfite radical (SO_3^-). This radical reacts with oxygen to form a sulfite peroxy radical ($\text{SO}_3\text{OO}\cdot$) and a sulfate radical (SO_4^-). These radicals in turn react with lipids resulting in the formation of lipid alkyl radicals [25, 26].

CONCLUSION

The present data confirm adverse effects of sulfites on the ovarian tissue due to its prooxidant activity. The progressive changes in many aspects of lifestyle, including dietary habits, have been shown to have detrimental effect on reproductive health. Therefore, these results are also relevant in terms of human exposure to sulfites especially in people who consistently eat processed or fast foods loaded with preservatives or in patients with congenital sulfite oxidase deficiency.

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