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SULPHUR DIOXIDE INDUCED OXIDATIVE AND HISTOPATHOLOGICAL DAMAGE TO THE OVARIES OF FEMALE RATS (*Rattus rattus*)

Prerna Sood* and Neena Singla

Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana - 140 000, Punjab (India)
*****e-mail: *prernasood@pau.edu*

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ABSTRACT

The aim of the study was to examine the toxic effect of sulphur dioxide on the ovaries of female rats. The effect of different concentrations of SO_2 (0, 5, 10 and 15 ppm) on antioxidant enzymes and histopathological changes were investigated in ovaries of female rats under natural and experimental conditions. Naturally exposed, house rats, *Rattus rattus* (Group I) were collected from agricultural fields alongside the road in Ludhiana, India and acclimatized for 1 month in laboratory. Laboratory-bred house rats were divided into four groups. Group II (control rats), III, IV and V were first exposed to the filtered air in 1 m^3 exposure chamber for 5 h day^{-1} for 28 days and then treated with 0, 5, 10 and 15 ppm of SO_2, respectively. SO_2 treatment showed significant increase in lipid peroxidation (LPO) levels, followed by significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST) and GSH-PX in ovaries of female rats. The results revealed that SO_2 can cause oxidative damage to the ovaries of female rats. The increase in LPO and decrease in other enzymes was more prominent at higher SO_2 concentration as compared to the other tested concentrations.

Keywords: Body weight, histopathology, oxidative damage, ovaries, rats, sulphur dioxide

INTRODUCTION

One of the largest problems in the majority of developing countries is the air pollution, mainly caused by industrial activity and the usage of fossil fuels. The air pollutants include particulate particles, sulphur dioxide (SO_2) and nitrous oxide (NO_2) (Ghorani *et al*., 2016). SO_2 pollution is more prominent in the areas surrounding coal-fired power plants, smelters and sulfuric acid industries as well as in densely populated areas. The respiratory tract may easily hydrate the inhaled SO_2 to form sulphuric acid, which then dissociates to form derivatives (bisulfite and sulfite) and enter into human body through blood (Shapiro, 1977). Substantial SO_2 exposures cause toxic symptoms such as thickening of mucous membrane lining the respiratory system, pneumonia, nasopharyngitis, fatiguability, gastritis and modifications in taste and smell. Numerous studies have reported the toxic effect of SO_2 on nervous system and reproductive system of mammals (Meng *et al*., 2002a.b, Meng 2003).

Very little information is available on how air pollutants including SO_2, NO_2 and particulate matters affect both male and female reproductive systems (Hansen *et al*., 2010). Inhaled pollutants or particles reportedly interfere in female reproductive system through the mechanisms of inducing oxidative stress in gonads, and by interrupting the maturation of germ cells by changing the expression of gene regulating differentiation or apoptosis in mice germ cells (Zhang *et al*., 2016; Wei *et al*.,

2017). SO_2 exposure in men and women may lead to some serious abnormalities like changes in sperm quality in men without changing the sperm numbers and changes in menstrual cycles, reduced ova production and infertility in women (Dejmek *et al*., 2000). Understanding the impact of environmental pollutants on human reproductive system has gained increasing attention. Reduced ovarian growth and altered levels of sex hormones are the effects of heavy metal exposure such as arsenic, lead, and mercury. Moreover, exposure to artificial organic substances like organochlorides and organo-phosphate in environment and at work lowers the sperm quality. Organophosphates are a class of pesticide that includes methyl parathion.

The "National Advisory Committee for Acute Exposure Guideline Levels (AEGL), USA for Hazardous Substances" under the Federal Advisory Committee Act has given acute exposure guideline level for SO_2 as 3 ppm for 10 h and 5 ppm for 5 h (Meng and Zhang, 2003). Based on this exposure limit three different concentrations of SO_2 were selected for the present study. The 5 and 10 ppm concentration of SO_2 are 10- and 20-fold higher than the typical urban concentration (0.5 ppm) and is likely to induce harmful effects to respiratory system of healthy individuals (Li *et al*., 2019). The concentration of 15 ppm which is beyond the natural exposure was used to examine the effects of higher SO_2 concentration on the health of individuals. In this study, the ovaries of female rats were examined for oxidative damage and stress and histopathological damage instigated by SO_2 inhalation at 3 levels (5, 10 and 15 ppm) and under field conditions.

MATERIALS AND METHODS

Mature apparently healthy female rats (*Rattus rattus*) of 100-150 g weight were collected from the agricultural fields alongside the road in Ludhiana (India) and acclimatized for 1 month in laboratory (group I). Also, the laboratory bred rats were procured and exposed to filtered air for 5 h day^{-1} for 28 days in 1 m^3 exposure chamber and then equally divided into four groups. Groups II, III, IV and V were exposed to 0, 5, 10 and 15 ppm SO_2 gas, respectively. Each group consists of 6 female rats. SO_2 gas was provided to treated rats through a tube located at the top of each chamber and was uniformly distributed with the help of a fan in each chamber (Meng and Zhang, 2003). Rats were kept in cages under standard conditions of humidity and temperature with light-dark cycle. Food (loose mixture of cracked wheat grains, powdered sugar and edible vegetable oil in ratio 96:2:2) and water were provided to control and treated rats *ad libitum*. The weight of individual rat was recorded weekly. After 28 days of exposure and deprivation of food for 18 h the rats were dissected. The experiment was performed with the ethical approval of Institutional Animal Ethical Committee, Guru Angad Dev Veterinary and Animal Sciences University Ludhiana, India vide communication No. GADVASU/2022/IAEC/64/17) dated March 11, 2022.

Measurements of antioxidant enzymes

After dissection, the ovaries of female rats were removed and weighed. The tissues were sheared in chilled saline and homogenized in phosphate buffer. The homogenates were centrifuged for 30 min at 1000 rpm at 4^0C to obtain supernatants. The tissue supernatants were used for the assay of different antioxidant enzymes like superoxide dismutase (SOD), glutathione S-transferase (GTS), Catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), glutathione reductase (GR) and lipid peroxidation (LPO), and proteins by standard method of Marklund and Marklund (1974), Habig *et al.* (1974), Hafeman *et al.* (1984), Carlberg and Mannervik (1985), Aebi (1983), Stocks and Dormandy (1971), Jollow *et al.* (1974), and Lowry *et al*. (1991), respectively. The enzymes SOD, GST, CAT, GPx, GSH, GR, LPO and proteins were expressed in terms of U mg^{-1} protein, µmole GSH-CDNB conjugate formed min^{-1} mg^{-1} protein, µmole H_2O_2 decomposed min^{-1} mg^{-1} protein, U mg^{-1} protein, µmole NADPH oxidized min^{-1} mg^{-1} protein, nmol MDA 100 mg^{-1} tissue, mg mL^{-1} tissue, respectively.

Histological studies

Histopathological studies were done according to the standard method of Luna (1968). Ovaries of female rats were cleared and fixed in 10% formalin for 24 h. Then the ovarian tissues were dehydrated in different grades of ethanol. The clearing was done in xylene while embedding was done in paraffin wax for the preparation of blocks. The 5-7 µm thick sections were cut, then stained in haematoxylin-eosin stain and mounted in DPX. The slides were observed under an optical light microscope (Magnus) using Magvision software for the histopathological changes against control slides. The photographs were also taken.

Statistical analysis

The data was subjected to analysis of variance (ANOVA) for making comparisons between control and treated group of rats (Meng, 2002a). The significant statistical difference was determined by selecting "p" value of 0.05.

RESULTS AND DISCUSSION

Both children and adults are impacted by SO_2 pollution which results in reduced work productivity and significantly high economic and social consequences for communities. In present study, 5, 10 and 15 ppm SO_2 concentrations were used to assess the effect of SO_2 inhalation on the ovaries of female rats in comparison to the control rats. The SO_2 concentrations of 5 and 10 ppm are 10 and 20 times higher than the normal urban concentration (0.5 ppm). The third concentration 15 ppm is above the level of natural exposure, and was evaluated to assess how the increased SO_2 concentration affects the health of people. SO_2 exposure was given for 5 h day^{-1} with relief periods between next exposure which is same as that of exposure of individuals working in industries or other occupational settings. The body weight and organ weight of the ovarie, oviducts and uterus did not vary significantly between the control and treated groups (Table 1). Table 2 presents the antioxidant activities of SOD, CAT, GST, GPx, GSH and GR in ovaries of female rats exposed to SO_2 at test concentrations (5, 10 and 15 ppm) and their corresponding control and naturally exposed rats. A non-significant decrease in SOD, CAT, GST, GR and GSH activities in rat ovaries was observed at 5 and 10 ppm SO_2 concentration. However, at 15 ppm SO_2 concentration significant decrease in SOD, CAT, GPx and GSH activities was noticed. A significant increase in lipid peroxidation (LPO) activity was observed at 15 ppm SO_2 concentration in rat ovaries of both the sexes as compared to the control. Higher SO_2 concentration decreased SOD, CAT, GST, GPx, and GSH activities but increased lipid peroxidation indicating the increased oxidative damage and stress in cells and tissues. These findings demonstrate that SOD and GPx activities were altered by SO_2 in rat ovaries in a dose-dependent manner. At low concentrations, the antioxidant enzymes remained significantly unaffected by SO_2, whereas at higher concentrations SO_2 caused a significant decrease in their activities. The fact that ovaries are susceptible to oxidative stress due to the oxygen free radicals (Chance, 2020). Since SOD catalyses the breakdown of superoxide radicals to form H_2O_2, the brain may be more susceptible to increased free radical damage as a result of antioxidant enzymes' decreased activity (Shen, 2020).

Table 1: Effect of SO_2 on the weights of reproductive organ (g 100 g^{-1} b.w.) of female rats

Name of organ	Naturally exposed rats	SO_2 concentration used (ppm)			
		Control (0)	5	10	15
Ovary	0.017 ± 0.007[a]	0.02 ± 0.001[a]	0.010 ± 0.001[a]	0.016 ± 0.003[a]	0.018 ± 0.004[a]
Oviduct	0.170 ± 0.003[a]	0.01 ± 0.004[a]	0.007 ± 0.001[a]	0.007 ± 0.0006[a]	0.007 ± 0.0006[a]
Uterus	0.010 ± 0.003[a]	0.01 ± 0.004[a]	0.007 ± 0.0005[a]	0.007 ± 0.001[a]	0.007 ± 0.0005[a]

Values expressed as mean ± SE;

[a]represents non-significant difference between treatments at $p \leq 0.05$ as compared to control

Table 2: Effect of SO_2 on antioxidant parameters of ovary of female rats

Enzymes	Naturally exposed rats	SO_2 concentration used (ppm)			
		0 (Control)	5	10	15
Superoxide dismutase (U mg^{-1} protein)	12.57 ± 0.15^{c}	13.26 ± 0.15^{c}	7.20 ± 0.47^{a}	7.68 ± 0.05^{a}	7.24 ± 0.48^{ab}
Catalase (μmole H_2O_2 decomposed min^{-1} mg^{-1} protein)	11.46 ± 0.03^{d}	12.90 ± 0.03^{d}	9.81 ± 0.38^{c}	8.86 ± 0.02^{b}	7.55 ± 0.24^{a}
Glutathione-S-transferase (μmole GSH-CDNB conjugate formed min^{-1} mg^{-1} protein)	0.43 ± 0.006^{b}	0.49 ± 0.001^{c}	0.43 ± 0.012^{b}	0.41 ± 0.002^{a}	0.42 ± 0.006^{a}
Glutathione peroxidase (U mg^{-1} protein)	0.91 ± 0.008^{b}	0.93 ± 0.01^{b}	0.96 ± 0.01^{b}	0.91 ± 0.008^{b}	0.86 ± 0.01^{a}
Glutathione (nM mg^{-1})	46.65 ± 0.16^{c}	48.65 ± 0.16^{c}	40.51 ± 0.48^{a}	41.60 ± 0.39^{a}	40.97 ± 0.58^{a}
Glutathione reductase (μmole NADPH oxidized min^{-1} mg^{-1} protein)	0.06 ± 0.05^{a}	0.06 ± 0.0009^{b}	0.06 ± 0.003^{a}	0.05 ± 0.0009^{a}	0.05 ± 0.001^{a}
Lipid peroxidase (nmol MDA 100 mg^{-1} tissue)	0.50 ± 0.01^{a}	0.49 ± 0.01^{a}	0.93 ± 0.01^{b}	0.56 ± 0.02^{a}	0.93 ± 0.01^{b}

Values expressed as mean ± SE;
^{abcd} represents significant difference between treatments at $p \leq 0.05$ as compared to control.

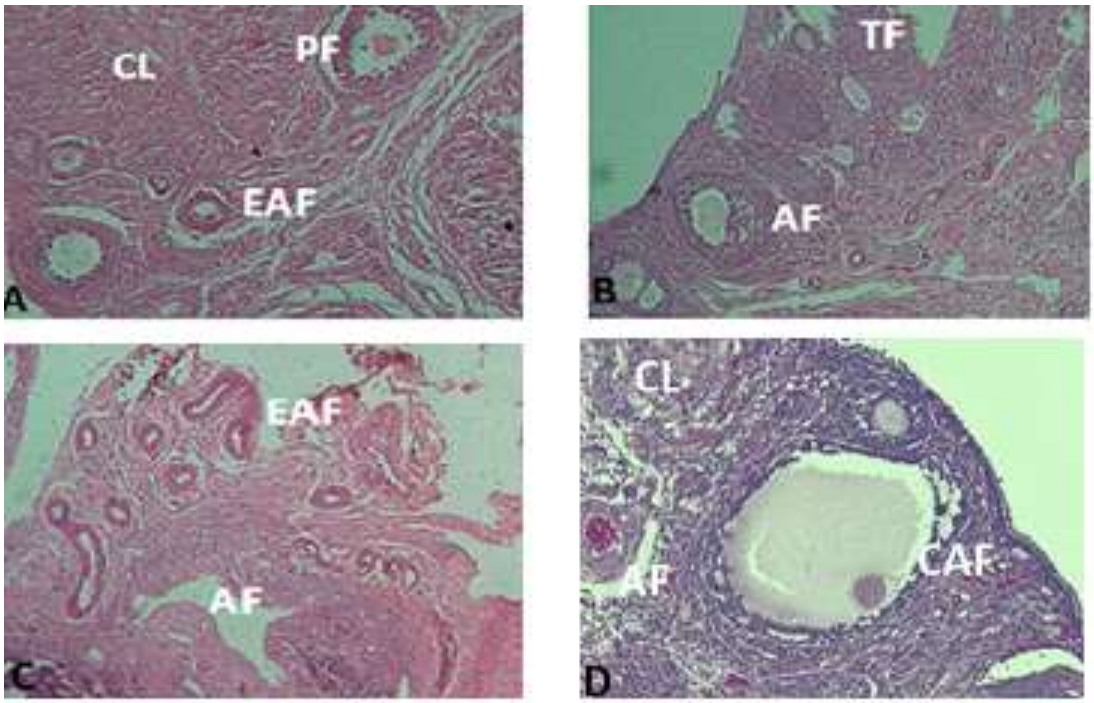

Fig. 1: Ovary sections; A) Primary follicles (PF), Corpus luteum (CL) and early antral follicles (EAF) in control rat; B) More tertiary follicles (TF) antral follicles (AF) in 5 ppm SO_2 exposed group; C) Antral follicles (AF) and early antral follicles (EAF) in 10 ppm SO_2 exposed group; and D) Corpus luteum (CL), increased number of antral follicles (AF), cystic antral follicle (CAF) and disruption of all stages of follicles in 15 ppm SO_2 exposed group.

SOD is thought of as the first line of defence against oxygen poisoning (Van Loon *et al*., 1986). According to Chance *et al*. (2020), se-dependent GPx and CAT are the main hydrogen peroxide

scavengers. Numerous studies have reported the toxic effect of SO_2 on the reproductive system and respiratory system of mammals and ovaries as sites for free radical generation and damage (Liu, 2022). Bakurt (1994) also reported decreased SOD level in the ovaries and lungs of rats after SO_2 exposure. Our findings are consistent with that of Medeiros (1983), who demonstrated that mice exposed to SO_2 experience a decrease in anti-oxidation status and an increase in lipid peroxidation.

Cell protection against free radicals is compromised by decrease in CAT and GPx levels. It is well known that glutathione peroxidase reduces hydroperoxides to water by oxidizing reduced glutathione to glutathione disulphide (GSSH). It has been discovered that the reduction in GST, GSH, and GPx levels at increased SO2 concentrations was dosage dependent. Similar decrease in GST, GSH, and GPx levels were also noted by Leung *et al*. (1985) and Lovati *et al.* (1996). Some other rat experiments also demonstrated a decreased level of GSH following SO_2 inhalation (Lovati *et al*. 1996). Under normal circumstances, antioxidant enzymes such as SOD, CAT, GSH, and GPx help cells combat free radicals (Etlik *et al*., 1995). Haider *et al*. (1981) have revealed elevated lipid peroxidation in rats' brains and guinea pigs' erythrocytes that were exposed to SO_2. Tyler (2018) also explained the enhanced lipid peroxidation in mitochondria caused by oxygen free radical stress following exposure to SO_2 derivatives in a lab setting. The toxic effect of SO_2 on female reproductive system, respiratory and nervous systems of mammals and ovaries as sites for free radical generation and damage have previously been reported (Dejmek *et al.*, 2000; Meng, 2002a; 2003).

The SO_2 has a greater impact on fecundability of men and women (Dejmek *et al*., 2000). Evidences demonstrate that air pollution contributes to the pathophysiology of female infertility (Mahalingaiah *et al*., 2016). It appears that air pollution is a cause of concern for mammalian health. Only three retrospective studies examined the impact of air pollution on *in vitro* fertilization (IVF). Despite studying a sizable IVF cohort, Legro *et al.* (2010) found two significant limitations *viz*., i) the variety of IVF techniques and ii) the lack of data on male partners. Further, Perin *et al*. (2010) have reported limitations due to the study on a single pollutant (SO_2) and the small number of cases of effect of SO_2 on female reproductive system. More studies are required to be done to assess the impact of SO_2 on reproductive organs of mammals and humans.

Conclusions: The SO_2 exposure caused a significant increase in lipid peroxidation process in rat ovaries of females that was supplemented by reduction in antioxidant status in a dose-dependent manner in laboratory rats as compared to the field rats. Secondly, the effects of SO_2 on mammals are multifaceted and it is a toxin to ovaries besides other organs. Further work needs to be done to understand the toxic role of SO_2 on all organs of mammals.

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Ethical statement: The present study was conducted after seeking ethical approval from the Institutional Animal Ethical Committee, GADVASU, Ludhiana, Punjab (India) vide their communication No. GADVASU/2022/IAEC/64/17) dated March 11, 2022.

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