# EFFECTS OF SODIUM FLUORIDE AND SULFUR DIOXIDE ON OXIDATIVE STRESS AND ANTIOXIDANT DEFENSES IN RAT TESTES

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**SUMMARY: To assess effects of sodium fluoride and sulfur dioxide on oxidative stress and antioxidant defenses in the testes, 96 sexually mature male Wistar rats were divided randomly into four groups of twenty-four rats each. One group of rats was left untreated as controls, and the other three groups were administered, respectively, for eight consecutive weeks, 100 mg NaF/L (45 mg $F^-$/L) in their drinking water, sulfur dioxide in ambient air (15 ppm $SO_2$, 4 hr/day), or were exposed to the same levels of both NaF and $SO_2$ together. In comparison with the control group, testis glutathione peroxidase (GSH-Px) activity significantly increased in the $SO_2$ group and in the NaF+$SO_2$ group in the 2nd and 6th week of exposure. Superoxide dismutase (SOD) activities also increased markedly in the NaF group at week 2, in the $SO_2$ group at weeks 2 and 6, and in the NaF+$SO_2$ group from week 6 to week 8. Increased malondialdehyde (MDA) levels occurred in the NaF group at week 6, in the $SO_2$ group at weeks 2, 6, and 8, and in the NaF+$SO_2$ group from week 4 to week 8. However, the ratios of SOD activity and MDA content in treated groups were lower than those of the control group, especially in the NaF+$SO_2$ group at week 6. These results suggest that oxidative stress from NaF and $SO_2$ in the testes may be one of the causes of reduced sperm motility in male rats.**

Keywords: Antioxidant defense; Fluoride and sulfur dioxide; Glutathione peroxidase; Male rats; Malondialdehyde; Oxidative stress; Rat testes; Sodium fluoride; Sulfur dioxide; Superoxide dismutase.

## INTRODUCTION

Fluoride, originating especially from industrial sources, is widely present in the environment—in water, soil, air, food, and vegetation with a significant increase in recent years in body burden accumulation. Sulfur dioxide ($SO_2$) is abundant in the environment as a primary airborne contaminant and is reported to increase the prevalence of dental fluorosis in coal-burning polluted fluorosis areas.[1-3] Our recent investigation[4] indicates that the combination of fluoride and $SO_2$ affects both sperm motility and reproductive hormone levels in male rats thereby causing considerable damage. It is therefore important to explore the mechanisms causing these changes in reproduction parameters of male rats exposed to fluoride and $SO_2$.

Superoxide free radicals and lipid peroxidation mechanisms play an important role in spermatogenesis.[5-7] Although the findings differ,[8] various studies in different organs of animals and humans also indicate that both fluoride [9-15] and $SO_2$[16-17] induce oxidative stress, respectively. However, there appear to be few reports on the effects of fluoride and $SO_2$ on oxidative stress in the testis. The present study, therefore, aimed to examine oxidative stress and antioxidant defense in the testis as they might relate to our recent investigations showing reduction of

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sperm motility in male rats administered either NaF or $SO_2$ alone or in combination.

## MATERIALS AND METHODS

*Experimental materials:* As in our recent study,[4] twelve-week old male Wistar albino rats, each weighing approximately 160 g, were obtained from the Experimental Animal Center of Shanxi Medical University along with their standard diets. The same pure $SO_2$ gas (99.99%) and the apparatus used previously [4] were employed here for this investigation.

*Establishment of animal model:* As in our recent report,[4] ninety-six of the above male rats were randomly divided into four groups of twenty-four: (1) a control group, which was given distilled water and clean air; (2) a sodium fluoride (NaF) group, to which 100 mg NaF/L (= 45 mg $F^-$/L) was administered in their drinking water; (3) a sulfur dioxide ($SO_2$) group, for which a 15 ppm $SO_2$ concentration in air was maintained continuously every day of each week for 4 hr from 8:00 am to 12:00 noon; and (4) a sodium fluoride and sulfur dioxide (NaF+$SO_2$) group which was administered the above treatments together. The F levels in the diet and drinking water, and the $SO_2$ concentration in the ambient air are shown in Table 1. All rats were maintained on normal diets under standard temperature (22–25°C), 12/12-hr light/dark cycle, ventilation, and hygienic conditions. At the end of weeks 2, 4, 6, or 8, six rats from each group were randomly selected for study.

**Table 1.** Fluoride levels in diet (mg/kg) and drinking water (mg $F^-$/L) and sulfur dioxide ($SO_2$) concentration in ambient air (ppm)

| | Control | NaF | $SO_2$ | NaF+$SO_2$ |
|---|---|---|---|---|
| Fluoride in diet | 23.39±1.04 | 23.39±1.04 | 23.39±1.04 | 23.39±1.04 |
| $SO_2$ in ambient air | <0.1[a] | <0.1[a] | 15.0±5.0[b] | 15.0±5.0[b] |
| Fluoride in drinking water | <0.6 | 45[c] | <0.6 | 45[c] |

[a]Sulfur dioxide gas in air could not be detected below 0.1 ppm with our gas monitor.

[b]15 ppm sulfur dioxide emission was maintained continuously for four hours from 8:00 am to 12:00 noon during every exposure day; ±5.0 indicates the maximum (20 ppm) and minimum (10 ppm) $SO_2$ concentration extremes, not standard deviation or standard error.

[c]From 100 mg NaF/L.

*Assay of lipid superoxides and antioxidant enzymes in testis:* At the end of weeks 2, 4, 6, and 8, six rats from each group were randomly selected and sacrificed by cervical dislocation, and the right testes were immediately collected and weighed and then homogenized with 1:9 (w/v) 0.9% saline solution at 0–4°C. Glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity, and the malondialdehyde (MDA) content in the testis tissue were determined with the reagent kit provided by the Nanjing Jianchen Biological Institute.

## RESULTS

*GSH-Px activity, SOD activity, and MDA content of the testis tissue:* The GSH-Px activity, total SOD activity, and MDA content in the right testis homogenates from the experimental rats according to their treatment are listed in Tables 2, 3, and 4, respectively. Table 5 shows the ratio of testis SOD activity to MDA content.

**Table 2.** GSH-Px activity in testis homogenates of male rats
(U/mg protein; mean ± SD; n = 6 in each group)

| Treatment weeks | Control | NaF | $SO_2$ | NaF+$SO_2$ |
|---|---|---|---|---|
| 2 | 10.08±0.57 | 11.95±0.68 | 13.07±1.09* | 12.70±0.88* |
| 4 | 11.02±0.35 | 11.26±0.39 | 11.46±1.17 | 11.24±0.57 |
| 6 | 5.83±0.34 | 6.53±0.73 | 7.80±0.19* | 8.24±0.44† |
| 8 | 9.04±0.71 | 8.90±0.31 | 7.22±0.86 | 10.02±1.59 |
| Mean value | 8.99±0.51 | 9.66±0.55 | 10.48±0.78 | 10.55±0.58 |

*P<0.05. †P<0.01 (compared with the control group).

**Table 3.** SOD activity in testis homogenates of male rats
(U/mg protein; mean ± SD; n = 6 in each group)

| Treatment weeks | Control | NaF | $SO_2$ | NaF+$SO_2$ |
|---|---|---|---|---|
| 2 | 88.43±2.15 | 108.49±5.59* | 113.52±6.36† | 95.99±6.59 |
| 4 | 81.83±2.52 | 80.72±6.27 | 76.96±6.12 | 89.16±2.66 |
| 6 | 49.52±5.85 | 52.82±5.54 | 74.45±1.85† | 71.35±2.08† |
| 8 | 64.09±7.00 | 59.97±2.93 | 71.03±2.24 | 95.43±8.10* |
| Mean value | 70.97±4.15 | 75.50±5.51 | 86.22±5.77* | 88.76±3.49* |

*P<0.05. †P<0.01 (compared with the control group).

**Table 4.** MDA in testis homogenates of male rats
(nmol/mg protein; mean ± SD; n = 6 in each group)

| Treatment weeks | Control | NaF | $SO_2$ | NaF+$SO_2$ |
|---|---|---|---|---|
| 2 | 83.82±11.55 | 95.24±4.27 | 126.10±10.67* | 113.03±10.58 |
| 4 | 80.20±2.55 | 111.18±12.12 | 111.45± 9.72 | 125.52±21.62* |
| 6 | 45.91±4.58 | 70.78±5.97* | 96.18±9.90† | 96.92±3.90† |
| 8 | 102.68±8.46 | 110.29±6.82 | 124.87±3.71 | 219.50±3.51* |
| Mean value | 74.12±7.11 | 96.87±5.20 | 115.82±5.11† | 138.56±15.99† |

*P<0.05. †P<0.01 (compared with the control group).

**Table 5**. SOD/MDA ratio in testis homogenates from male rats
(mean ± SD; n = 6 in each group)

| Treatment weeks | Control | NaF | $SO_2$ | NaF+$SO_2$ |
|---|---|---|---|---|
| 2 | 1.14±0.17 | 1.15±0.08 | 0.91±0.05 | 0.89±0.12 |
| 4 | 1.02±0.04 | 0.77±0.11 | 0.70±0.06* | 0.78±0.12 |
| 6 | 1.08±0.24 | 0.75±0.05* | 0.77±0.06* | 0.74±0.05* |
| 8 | 0.62±0.03 | 0.55±0.05 | 0.57±0.10 | 0.49±0.08 |
| Mean value | 0.97±0.12 | 0.81±0.06 | 0.75±0.04* | 0.74±0.06* |

*P<0.05 (compared with the control group).

## DISCUSSION

Many investigations indicate that excessive fluoride (F) or sulfur dioxide ($SO_2$) can enhance lipid peroxidation and inhibit antioxidative enzymes in various organs of animals.[9-12,14,15,17] However, in the present study, dynamic observations show no significant change in GSH-Px activity in the testis in the NaF group during the entire 8-week period as compared with the control, and yet GSH-Px activity significantly increased in the $SO_2$ group and in the NaF+$SO_2$ group of male rats in the 2nd and 6th week of exposure. Similarly, an increase in SOD levels in testis tissues was monitored at various stages during the process of spermatogenesis in the NaF+$SO_2$ group. Thus testis SOD activities significantly increased in the NaF group at week 2, in the $SO_2$ group at weeks 2 and 6, and in the NaF+$SO_2$ group from week 6 to week 8. The differences might be due to variation effects in the dose, the life stage, exposure duration, and route of F and $SO_2$ administration, the particular animal species used, or individual tissue response. It is also possible that a positive feedback mechanism in response to increased lipid peroxidation may be responsible for the activation of these antioxidant enzymes.

Malondialdehyde (MDA) is considered an index of lipid peroxidation as demonstrated by many earlier studies revealing increased MDA levels in the testis of animals that have been individually exposed to F or to $SO_2$.[18,19] In the present study, the testis MDA level increased significantly in the NaF group at week 6, in the $SO_2$ group at weeks 2, 6, and 8, and in the NaF+$SO_2$ group from week 4 to week 8, as compared with that of the control group. In particular, the mean value of the evaluated period was slightly higher in the NaF group, but was markedly higher in the $SO_2$ group and in the NaF+$SO_2$ group. The above findings indicate that $SO_2$ and the interaction of F and $SO_2$ can result in a more significant change in lipid peroxidation than F alone.

It should also be noted that the increase in both SOD and GSH-Px activity paralleled the increased MDA content in the same group at various stages of spermatogenesis as we reported recently.[20] Therefore, we agree with the opinion that changes in the activity of these antioxidant enzymes may be an adaptive reaction to changes in the corresponding lipid peroxides. However, the fact remains that the ratios of SOD activity and MDA content in treated groups were

lower than those of the control group, especially in the NaF+$SO_2$ group at week 6. From this result it is apparent that the increase in lipid peroxidation caused by F and $SO_2$ exposure generally resulted in a decrease in the antioxidant ability, with the result that various types of lesions were more likely to emerge in the testes.

Likewise, germ cells, in comparison to somatic cells, are more susceptible to oxidative stress. There are two reasons for this. First, they are intimately associated with the free radical-generating phagocytic Sertoli cells,[6] and, second, germ cell plasma membrane contains a higher amount of polyunsaturated fatty acids that are vulnerable to oxidation by free radicals.[21] Hence, sperm cell membrane repair is induced by oxidative stress in the testis, which may result in inhibition of testicular spermatogenesis and a reduction in sperm activity.

In conclusion, oxidative stress induced by F and $SO_2$ should be considered as one of the pathways that lead to reduction in sperm activity in male rats.

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