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Original Article

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Effects of Air Pollution, Ozone, and Sulfur Dioxide on Kidney Oxidative-Stress Enzymes, Histopathology, and Apoptosis Gene Expressions in Rats

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ABSTRACT

Background: Ambient air pollution (AAP) is an essential global problem that affects most of the body's organs, including the kidneys. We investigated the effects of AAP, sulfur dioxide (SO_2), and ozone (O_3) on kidney functions, histology, antioxidant enzymes, and apoptosis gene expressions in Wistar rats.
Methods: The rats were divided into 4 groups (n = 8): control, AAP, SO_2 (10 ppm), and O_3 (0.6 ppm) groups. Blood urea and creatinine, kidney tissue superoxide dismutase, total antioxidant capacity, glutathione peroxidase activities, and malondialdehyde were determined after exposure to AAP, SO_2, or O_3 for 3 hours per day for 5 weeks. Kidney tissue was examined by light microscopy, and the expression of BCL-2, BAX, and caspases-3,-8, and caspase-9 were evaluated by the reverse transcription quantitative polymerase chain reaction technique.
Results: Mild to moderate histopathological changes in AAP and moderate-to-severe changes in SO_2 and O_3 groups were reported. Increment of serum urea in SO_2 and AAP groups ($P = .017$ and $P = .045$), kidney catalase in AAP group ($P = .008$), total antioxidant capacity in O_3 group ($P < .001$), caspase-8 expression in SO_2 group ($P = .035$), caspase-8, and caspase-9 expressions in O_3 group ($P < .001$ and $P = .003$) were significant. p53 expression in the kidney of the AAP group was less than that of the control group ($P = .018$).
Conclusion: Ambient air pollution, SO_2, and O_3 exposures in the concentrations used in our study cause kidney dysfunction, stimulate oxidative stress defense enzymes, cause histopathological changes, and downregulate kidney p53 expression.
Keywords: Air pollution, apoptosis, caspases, kidney, ozone, sulfur dioxide

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INTRODUCTION

Ambient (outdoor) air pollution (AAP) is a mixture of suspended particles and gases whose concentrations harm humans.[1] Air pollutants can refer to suspended particles, chemicals, including stable organic pollutants, and gas compounds such as ozone (O_3), sulfur oxides (SO_x), carbon monoxide (CO), nitric oxides (NO_x), and heavy metals. Particulate matter (PM) is a complex mixture of chemical compounds that are different in size. $PM_{2.5}$ is a category of particulate pollutants that are 2.5 µm or smaller in size. Sulfur dioxide (SO_2) is one of the primary air pollutants, mainly produced by the combustion of fossil fuels that contain sulfur. Nitric oxides, CO, and volatile organic compounds following a series of photochemical reactions can lead to O_3 formation in the troposphere. Ozone gas in the troposphere and in the ground level can have adverse health effects.[2]

Ambient air pollution, as a significant environmental health problem, affects most of the world's industrialized and populous countries as the urban population increases and ecological pollutants rise.[3,4] Ambient air pollution adversely impacts kidney function, respiratory, cardiovascular, and nervous systems and has been

considered the cause of many non-communicable diseases.[2,5-7] The positive association between increased risk of acute kidney injury and chronic kidney disease (CKD) and exposure to AAP has been suggested by shreds of evidence from animal and epidemiological studies.[1,7]

It is not precisely known which air pollutants cause explicit kidney damage by themselves and what type of damage they are responsible for. Evidence suggests that short-term exposure to environmental SO_2 may cause systemic oxidative stress and inflammation.[2] The hypothesis of kidney damage by air pollutants may be explained as follows: The inflammatory mediators induced by contaminants in the respiratory system can result in systemic oxidative stress, inflammation, and subsequent damage to kidney tissue and other distant organs. Oxidative stress overwhelming the endogenous antioxidant defenses may result in cellular dysfunction and damage. The pollutants translocated into the circulation via the pulmonary epithelium may cause oxidative stress, which is known to cause vascular disorders[1,8] although the pathogenetic mechanism is still unclear. More investigations are recommended to pinpoint the type of pollutant and how it is primarily responsible for kidney disorders.[1]

The gap in significant studies evaluating the effects of AAP and its components on kidneys and the pathogenic mechanisms underlying the effects has led us to design and perform the study. In the present study, we investigated the effects of AAP, SO_2, and O_3 (as AAP components) on kidney function and histopathology, oxidative stress parameters, including total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and malondialdehyde (MDA), and some apoptosis genes mRNA expressions in a rat model.

MATERIAL AND METHODS

The experiments were performed on adult Wistar male rats (180-220 g) purchased from the Pastor Institute (Karaj, Iran). The rats were housed in special cages in a room and fed a standard diet. The rats were maintained under a 12-hour light–12-hour darkness cycle with a constant temperature (22 ± 2°C) and humidity (40%-60%), and had free access to water and food.

MAIN POINTS

- Air pollution exposure results in kidney histopathological changes and blood urea enhancement.
- Sulfur dioxide and ozone exposures result in severe kidney histopathological changes and blood urea enhancement.
- Air pollution, sulfur dioxide, and ozone exposures can stimulate kidney oxidative defense enzymes.
- Sulfur dioxide and ozone exposures have extrinsic apoptotic pathways that trigger effects on the kidney tissue.
- Air pollution exposure has a downregulating effect on p53 expression in kidney tissue, which may help predispose to tumor progression.

Ethical Approval

The experiments were performed according to the guidelines of the research ethics committee of Tabriz University of Medical Sciences (Approval no: IR.TBZMED.VCR.REC.1399.037; Date: 2020.05.18). National Institutes of Health guidelines for the use of laboratory animals were considered in the procedures.

Procedures

The rats were randomly divided into 4 groups (n = 8 in each group) as follows:

- The control group: rats in this group were placed under filtered air conditions only. High-efficiency particulate air (HEPA) and activated carbon filters (Panam Azma, Iran) were used for the filtration of air. The levels of the pollutants (SO_2, O_3, and $PM_{2.5}$) of the control group were at undetectable levels.
- The SO_2 group: rats were exposed to 26 mg/m^3 SO_2 (10 ppm) for 5 weeks (3 hours per day). A handmade glass chamber ($40 \times 50 \times 60$ cm^3) equipped with bottom and top vents was used. Two 50 L SO_2 cylinders (120 bar pressure) with a flow rate of 1 L/min were used to provide the exposure chamber's SO_2. The composition of the gases in the chamber was nitrogen (N_2) (79.5%), O_2 (20.5%), and SO_2 (0.002%). An SO_2 detector tube with a measuring range of 2-30 ppm (GASTEC No.5La) was used for the daily detection of the chamber SO_2 concentration.
- The O_3 group: the rats were exposed to O_3 (1.18 ± 0.18 mg/m^3; 0.65 ± 0.15 ppm) in the chamber described previously for 5 weeks (3 h/day). An O_3 generator system (Afra Sanat, Iran) produced the O_3. The chamber's O_3 concentration was measured by a detector (Eco Sensors, model A-21ZX-USA) every day.
- The AAP group: rats were placed in a high-traffic city square of the city center, close to the air pollution recorder station, for 3 hours a day for 5 weeks.[9] Air pollution reported parameters during the investigation period are shown in Table 1.[10]

Twenty hours after the last intervention, the rats were anesthetized with ketamine (60 mg/kg IP) and xylazine (10 mg/kg IP), and blood samples were collected. The blood serums were separated and kept at −70°C. The left and right kidneys were taken and placed in a formalin fixative solution.

Biochemical Analysis

The blood serum urea and creatinine levels were assayed by commercial kits (Pars Azmoon, Karaj, Iran). Tissue samples (100 mg) were homogenized in the extraction buffer (1 mL) and centrifuged (10000 *g*, 10 minutes). The supernatants were separated and used for biochemical tests. The obtained biochemical values were corrected by the supernatant protein contents (Lowry assay). Cayman commercial kit (USA) and the colorimetric thiobarbituric acid method were used for catalase activity and MDA assays, respectively. The supernatant activities of

Table 1. Ambient Air Pollution Pollutants' Concentrations (Mean ± SD) During 5 Weeks of Study

Parameter	SO_2	O_3	CO	NO_2	$PM_{2.5}$	PM_{10}
Concentration in ambient air ($\mu g/m^3$)	2.00 ± 1.17	49.76 ± 18.48	14.08 ± 10.07	23.96 ± 8.26	28.67 ± 4.04	21.33 ± 5.13
WHO Guideline Values (upper limit) ($\mu g/m^3$)[12]	20 (24 hours' mean)	100 (8 hours' mean)	-	40 (annual mean)	10 (annual mean)	20 (annual mean)

CO, carbon monoxide; NO_2, nitrous dioxide; O_3, ozone; PM, particulate matter; SO_2, sulfur dioxide.

SOD, GPx, and TAC were colorimetrically assayed using ZellBio commercial kits (Ulm, Germany).

Histopathological Study

The formalin-fixed kidney samples were sectioned, processed, and stained with hematoxylin and eosin and examined using an Olympus microscope (Tokyo, Japan). The kidney pathological specimens were examined for tubular epithelium degeneration, tubular casts, vascular congestion, hemorrhage, necrosis, and interstitial fibrosis.[11] Four microscopic scores were considered for all parameters consisting of normal (0), mild (+1), moderate (+2), and severe (+3).

Reverse Transcription Quantitative Polymerase Chain Reaction

The reverse transcription quantitative polymerase chain reaction (RT-qPCR) method was performed to measure changes in the expression of some genes associated with apoptosis and survival, including BAX, p53, BCL-2, and caspases (3, 8, and 9). Thirty milligram of kidney tissue was used for total RNA extraction using a commercial extraction kit (NucleoSpin, Germany), and cDNA was synthesized (REVERTA-L RT kit, Moscow, Russia). The RT-qPCR assay was carried out using Applied Biosystems' StepOnePlus™ (USA), and 2-fold SYBR Green premix (Amplicon, UK) were used for the RT-qPCR assay. The primer sequence list for RT-qPCR is shown in Table 2. The expression of each group was stated as a mean decrease or increase in comparison with the control group.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences Statistics software for Windows, version 19 (IBM SPSS Corp.; Armonk, NY, USA). Initially, the variables were statistically checked for normality by the one-sample Kolmogorov–Smirnov test. All numerical variables had normal distributions and were presented as mean ± standard deviation (SD). The data between the groups were compared with a one-way analysis of variance, followed by the Tukey's post hoc test. The overall significance of the study was assessed at $P < .05$ and $P < .01$.

RESULTS

Blood Serum Biochemical Analysis

The mean urea and creatinine concentrations of blood serum samples in groups are shown in Figure 1A and Figure 1B. The blood urea levels of the SO_2 and AAP groups was significantly higher than the control group ($P = .017$ and $P = .045$, respectively). The AAP group's creatinine was significantly less than the O_3 and control groups ($P = .018$ and $P = .003$).

Histopathological Results

In the control group, a typical kidney parenchymal structure, together with well-defined glomeruli and tubules (score: 0), was observed. In contrast, the AAP recipient group presented mild (score: +1) to moderate (score: +2) pathological changes. However, in both O_3 and SO_2 recipient groups, there were moderate (score: +2) to severe (score: +3) pathological lesions. Indeed, there was a considerable difference in the air pollution recipient group compared to the O_3 and SO_2 recipient groups. Microscopically, the observed lesions included tubular epithelium degeneration, focal necrosis, vascular and glomerular congestion, and hemorrhage. Notably, focal mild (score: +1) to moderate (score: +2) inflammation was found in the O_3 and AAP recipient groups. Besides, a small number of tubular casts

Table 2. List of Reverse Transcription Quantitative Polymerase Chain Reaction Primer Sequences

Names of Genes	Forward Primer (5′ to 3′)	Reverse Primer (5′ to 3′)
BAX	AAGTGCCCGAGCTGATCAGAA	TGGGGGTCCCGAAGTAGGAAA
BCL-2	ATCGCTCTGTGGATGACTGAGTAC	AGAGACAGCCAGGAGAAATCAAAC
Caspase-3	ATGGACAACAACGAAACCTC	TTAGTGATAAAAGTACAGTTCTT
Caspase-8	CTGGGAAGGATCGACGATTA	CATGTCCTGCATTTTGATGG
Caspase-9	AGCCAGATGCTGTCCCATAC	CAGGAGACAAAACCTGGGAA
p53	CGGCCCATCCTTACCATCATC	CAGGCACAAACACGAACCTCA
GAPDH	CCCATCACCATCTTCCAGGAG	GAAGGGGCGGAGATGATGAC

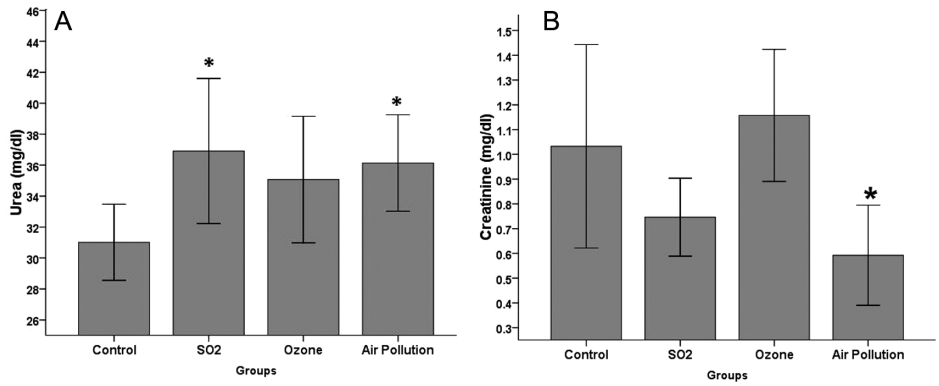

Figure 1. Effects of SO_2 (26 mg/m^3), ozone (1.18 mg/m^3), and ambient air pollution inhalation on blood urea (A) and creatinine (B) of the rats (mean ± SD; n = 8). *Significant differences with the control group at $P < .05$ level. SO_2, sulfur dioxide.

(score: +1) were detected in the O_3 group. There was no fibrosis (score: 0) in all 3 experimental groups (Figure 2).

Oxidative Stress Biomarkers

The mean kidney TAC of the O_3 group was significantly ($P < .001$) higher than the SO_2 and control groups (Figure 3A). As shown in Figure 3B, the GPx and SOD activities of kidney samples of the SO_2, O3, and AAP groups were higher than the control group ($P = .63$, $P = .18$, and $P = .111$, respectively for GPx and $P = .77$, $P = .756$, and $P = .331$ respectively for SO_2). Figure 3C shows that the kidney tissue catalase of the air pollution group is significantly higher compared to the control group ($P = .008$). The mean MDA concentrations of kidney tissue samples in groups are shown in Figure 3D. The kidney MDA of the SO_2 and AAP groups was significantly less than that of the control group ($P = .02$ and $P = .05$).

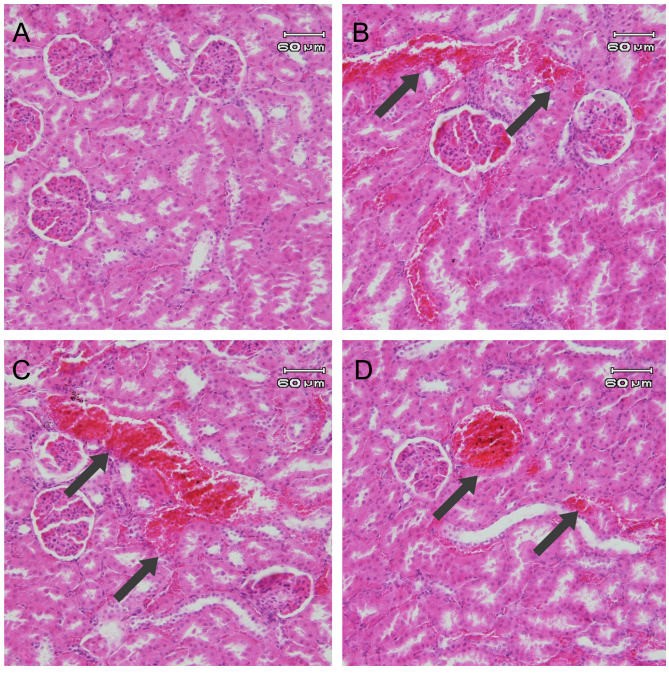

Figure 2. Kidney, rat. (A) The control (healthy) group with a typical kidney structure presented a normal glomerulus and tubule system. (B) Ozone group with severe (+3) histopathological changes with severe hemorrhage (arrows); (C) Sulfur dioxide group with severe (+3) histopathological changes with severe hemorrhage (arrows); (D) Ambient air pollution group with moderate (+2) histopathological changes, including moderate congestion and hemorrhage (arrows) (hematoxylin and eosin).

Reverse Transcription Quantitative Polymerase Chain Reaction Findings

The AAP group's p53 protein expression was significantly lower than that of the control and O_3 groups ($P = .018$ and $P = .044$, respectively). Expression of p53, BAX, and BCL-2 transcripts in the kidney tissue samples are shown in Figure 4. The AAP group's BAX protein expression was significantly higher than that of the SO_2 and O_3 groups ($P = .001$ and $P = .002$, respectively). BCL-2 protein expression of the O_3 group was higher than that of the other groups ($P = .174$).

Expression changes of caspases (3, 8, and 9) transcripts in kidneys are presented in Figure 5. Caspase-8 expression of the SO_2 group was significantly higher than that in the control group ($P = .036$). Caspase-8 expression of the O_3 group was considerably higher than that in control and AAP groups ($P < .001$ and $P = .002$, respectively). Caspase-9 expression of the O_3 group was significantly higher than that of the control, SO_2, and AAP groups ($P = .003$, $P = .007$, and $P = .013$, respectively).

DISCUSSION

Air Pollutants

The effects of AAP and its components on the kidneys and the pathogenic mechanisms are not clear enough. Our aim was to examine the effects of AAP on kidneys in a rat model. Polluted air compositions vary in different places, at other day times, and during the week due to amounts of air pollutants and various sources and conditions. We tried to standardize the pollutant levels as described previously. The mean ambient air SO_2,

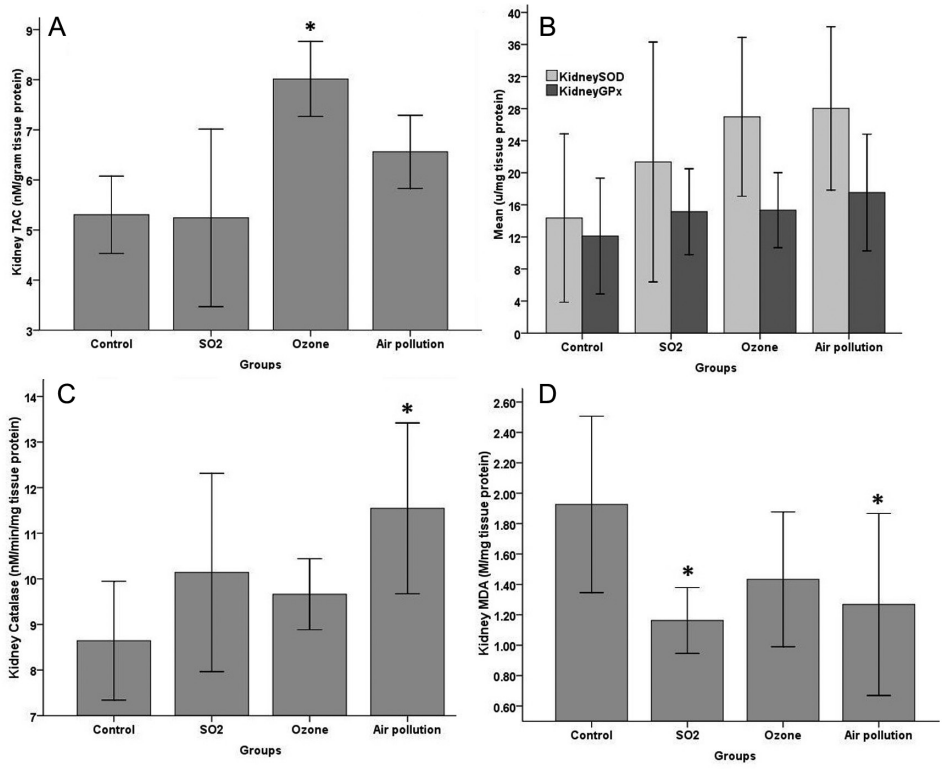

Figure 3. Effects of SO_2 (26 mg/m^3), ozone (1.18 mg/m^3), and ambient air pollution inhalation on kidney tissue TAC (A), SOD and GPx (B), catalase (C), and MDA (D) of the rats (mean ± SD; n = 8). *Significant differences with the control group at $P < .05$ level. GPx, glutathione peroxidase; MDA, malondialdehyde; SO_2, sulfur dioxide; SOD, superoxide dismutase; TAC, total antioxidant capacity.

O_3, and NO_2 concentrations during the study did not exceed the values of the WHO guideline,[12] but $PM_{2.5}$ and PM_{10} concentrations were higher than the values (Table 1).

Effects on Blood Serum Urea and Creatinine

Significant increments of the serum urea after SO_2 and AAP exposures indicate that kidney function was affected by them. Urea is an endogenous product of amino acid and protein catabolism during normal physiological processes.[13] A significant reduction in the serum creatinine level of the AAP group compared to the control group was unexpected, and the differences between other groups were not statistically significant. Creatinine is a by-product resulting from creatine catabolism.[13] There are limited data about the relationship between AAP and renal functions. Kuzma et al found the harmful effects of NO_2, SO_2, and PM on kidney function.[7] Gao et al. indicated that 4 weeks' exposure to ambient $PM_{2.5}$ could be strongly associated with a decrease in estimated glomerular filtration rate but could not be associated with blood urea nitrogen and odds of CKD.[21]

Feng et al. found no association between serum creatinine and CKD with $PM_{2.5}$ exposure levels.[22] As shown in our findings, surprisingly, the creatinine changes in SO_2 and O_3 groups were not significant, and in the AAP group, the creatinine level was significantly less than in the control group.

The difference between the trends of change in urea and creatinine levels in our study cannot be explained easily. This may be related to the small sample size or related to changes in the muscle mass that we did not have the opportunity to measure. Further investigations are needed, and we hope to explore that in our subsequent studies.

Effects on Kidney Histopathology

The results of the histopathological study showed mild-to-moderate changes in the AAP group, while moderate-to-severe changes, including tubular epithelium degeneration, focal necrosis, vascular and glomerular congestion, and hemorrhage, in the SO_2 and O_3 groups.

These findings are noticeable, considering that during the AAP exposition in our study, the $PM_{2.5}$ concentrations of AAP were higher than the WHO-limited value. It seems that the severity of histopathological changes in the SO_2 and O_3 groups is due to differences in the concentrations of SO_2 and O_3 in AAP, SO_2 and O_3 groups (2.0 and 49.8 μg/m^3 SO_2 and O_3 in the AAP vs. 26.0 and 1.18 mg/m^3, in the SO_2 and O_3 groups, respectively). Particulate matter contains chemicals, including heavy metals, which potentially contribute to systemic inflammation, which harms kidney function.[14] Some previous studies found that chronic or subchronic exposure to $PM_{2.5}$ may result in histopathological alterations in the tissue of the kidney and kidney

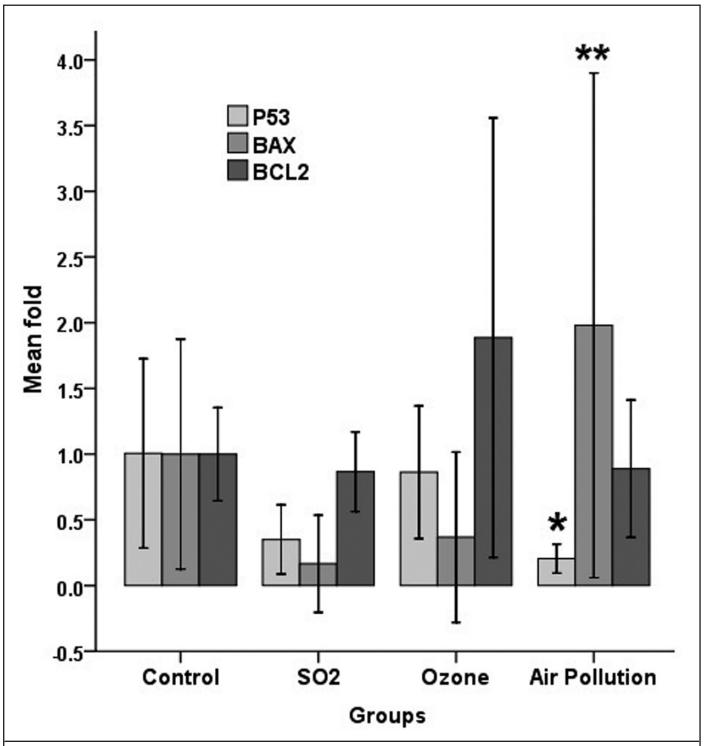

Figure 4. Expression of mRNA encoding proteins of p53, BAX, and BCL-2 in the kidney samples. *Statistically significant compared to the control and ozone groups (P = .018 and P = .044, respectively). **Statistically significant compared to the SO_2 and O_3 groups (P = .001 and P = .002, respectively). mRNA, messenger ribonucleic acid.

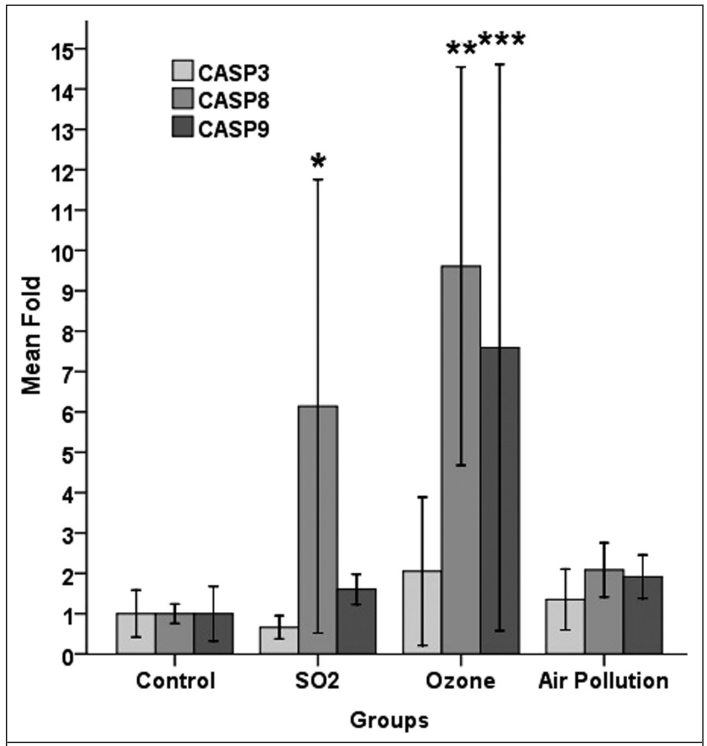

Figure 5. Expression of mRNA encoding caspase-3, caspase-8, and caspase-9 in the kidney samples. *Statistically significant compared to the control and O_3 groups (P = .036); **statistically significant compared to the control and AAP groups (P < .001 and P = .002, respectively); ***statistically significant compared to the control, SO_2, and AAP groups (P = .003, P = .007, and P = .013, respectively). mRNA, messenger ribonucleic acid.

dysfunction.[7,15,16-21] Tavera Busso et al[21] (2018) demonstrated that subchronic exposure to urban $PM_{2.5}$ might induce mesangial expansion, fibrosis, decreased tubular and glomerular lumen volumes, and blood urea elevation. A study on diabetic rats showed a positive association between glomerulosclerosis and exposure to ambient PM (13.3 µg/m^3 for 16 weeks).[1]

Effects on Oxidative Stress Biomarkers

An imbalance between free radicals (oxidants) and antioxidant systems creates oxidative stress. Cells can tolerate mild oxidative stress, but in more severe cases, the cell membrane is damaged, and subsequent pathological complications occur. To prevent such damage, enzymatic and nonenzymatic antioxidants are activated to remove reactive oxygen species (ROS) stimuli and allow the organism to withstand and overcome oxidative stress in contaminated environments. The enzymatic antioxidant defense system includes necessary intracellular antioxidant enzymes such as catalase, GPx, and SOD in cells to prevent uncontrolled ROS. Superoxide dismutase activity is often considered a biomarker of good pollution. This enzyme responds to environmental stress in a short time.[22]

Although our findings showed the enhancement effects of all exposures on kidney oxidative defense enzymes, including TAC, SOD, GPx, and catalase, only the effects of O_3 on TAC and AAP on catalase of kidney were statistically significant. The elevation of the mentioned parameters may be because of the stimuli effects of the O_3 and air pollutants exposures in the concentrations used in our study. Our findings are consistent with the previous results, which indicate that O_3 activates the antioxidant defense system through oxidative preconditioning.[23]

On the other hand, a significant reduction in kidney MDA levels was shown after the SO_2 and AAP exposures. MDA is a by-product of ROS-induced unsaturated fatty acid oxidation, studied as a biomarker of oxidative stress.[24] In our study, decrement of MDA levels can result from the reduction of lipid peroxidation, which may be a result of oxidative stress reduction. The kidney tissue oxidative stress reduction may result from kidney oxidative stress defense enzyme stimuli. Recent findings indicated that diesel exhaust exposure led to kidney oxidative stress, DNA damage, and kidney blood flow reduction.[25]

The literature review indicates the preventive effect of O_3 on lipid peroxidation. It has been reported that O_3 can form minimal amounts of lipid peroxidation products, resulting in an adaptive response in the cells that results in buffering mechanisms. It seems that buffering mechanisms are responsible for a significant reduction in patients' MDA levels after O_3 exposure. The substantial decrement in lipid peroxidation observed in our

study may be explained by the dominance of catalase and SOD activities over lipid peroxidation.[26]

Gutteridge and Halliwell[27] reported that an antioxidant's primary functions are to prevent oxidant formation, to scavenge ROS, and to repair oxidized molecules. In our findings, stimulation of kidney oxidative stress defense enzymes may result from neutralizing, followed by repairing phases. The induction of oxidative stress defense enzyme activities may be due to the activation of nuclear factor kappa B by H_2O_2. Nuclear factor kappa B, as a transcription factor, is involved in cellular stress responses, cycle regulation, inflammation, and apoptosis.[23] The tissue oxidative stress and antioxidant enzyme contents, as well as the severity of histopathological lesions can be affected by the kidney tissue sampling time after the intervention.

Altogether, our findings demonstrate that the AAP, SO_2, and O_3 exposures, in the concentrations used in our study, don't have considerable oxidative stress induction effects on kidney tissue but can stimulate kidney oxidative stress defense enzymes.

Effects on Kidney mRNA Expressions

Our study showed that caspase-8 expression increased in the SO_2 group, while caspase-8 and caspase-9 expression increased in the O_3 group. Caspases are proteases that exist as inactive zymogens in cells and undergo a cascade of catalytic activation at the onset of programmed cell death (apoptosis).[28]

The role of SO_2 in inducing cellular apoptosis, directly or indirectly, by increasing the interactive property of benzo(a)pyrene, was demonstrated in previous studies.[24] A synergistic effect of PM_{10} and SO_2 on overexpression of reduced cell survival and apoptotic conditions has been shown previously. While at the same concentrations of PM_{10} and SO_2, the cells were not damaged.[24]

In our study, a significant decrease in p53 in the kidney compared with the control group resulted after the AAP exposure ($P = .018$). In contrast, the effects of AAP, SO_2, and O_3 on kidney BAX and BCL-2 were not statistically significant. p53 protein acts as a tumor suppressor. p53 can induce senescence and programmed cell death. Recent studies strongly implicate a tumor suppression role for p53, particularly concerning metabolism control and iron- and lipid peroxide-mediated cell death. Inhibition of GPx4 by p53 is the crucial driver of the mentioned function.[26] BAX protein acts as a critical protein in apoptosis induced by various factors in the internal apoptosis pathway and can be directly regulated by p53. BCL-2 has an antiapoptotic effect in responding to different apoptotic stimuli by preventing the release of cytochrome C from mitochondria. Hsu et al[29] showed that exposure to AAP-related PM for 3-6 months could affect kidney function and induce autophagy and apoptosis in kidney tissues. Their results suggest that AAP may cause autophagy and nephrotoxicity and play a protective role in PM-induced cytotoxicity. Our results suggest that AAP, at our study concentrations, has a downregulating effect on p53 expression in kidney tissue, which may predispose to tumor progression and chemoresistance.[30]

Although SO_2 and O_3 are the components of AAP, we believe that their different effects on the assessed gene expressions in the present study are due to differences in SO_2 and O_3 concentrations in the groups and also the existence of substances other than SO_2 and O_3 in AAP such as PM, NO_2, and heavy metals.

Our findings demonstrated that the kidney is affected by the pollutants in the air, as shown by biochemical analysis and histopathological examination of the kidney tissue. Besides, oxidative stress, apoptotic pathways, and RNA expression seem to be altered with exposure to air pollutants. Further studies are needed to fully elucidate the effects of the mentioned pollutants, considering that more people are exposed to air pollution each year in the modern world.

Ethics Committee Approval: Ethics committee approval was received from the Ethics Committee of Tabriz University of Medical Sciences (Approval no: IR.TBZMED.VCR.REC.1399.037; Date: 2020.05.18).

Informed Consent: N/A.

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Declaration of Interests: The authors have no conflict of interest to declare.

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