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Tobacco smoke induces oxidative stress and alters pro-inflammatory cytokines and some trace elements in healthy indoor cats

Berjan Demirtas[1]*, Karolin Yanar[2], Lora Koenhemsi[3], Nurcan Erozkan Dusak[4], Ozlem Guzel[5], Alev Akdogan Kaymaz[3]

[1] Department of Plant and Animal Production, Equine and Training Program, Vocational School of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye; [2] Department of Medical Biochemistry, Cerrahpaşa Faculty of Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye; [3] Department of Internal Medicine, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye; [4] Central Laboratory, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye; [5] Department of Surgery, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, Istanbul, Türkiye.

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Abstract

This study was aimed to assess oxidative stress, pro-inflammatory cytokines and some trace elements in healthy pet cats exposed to environmental tobacco smoke. Forty healthy cats were included in this study. Cats were divided in two groups: Exposed to tobacco smoke (ETS; n = 20) and non-exposed to tobacco smoke (NETS; n = 20). Blood levels of cotinine, total oxidant status (TOS), oxidative stress index (OSI), lipid hydroperoxide (LOOH), protein carbonyl (PCO), advanced oxidative protein products (AOPP), total antioxidant status (TAS), copper, zinc-superoxide dismutase (Cu, Zn-SOD), catalase (CAT), total thiol (T-SH), interferon gamma (INF-γ), tumor necrosis factor (TNF-α), interleukin β (IL-1β), interleukin 6 (IL-6), interleukin-8 (IL-8), interleukin 2 (IL-2) and iron (Fe), zinc (Zn), copper (Cu), selenium (Se) levels were measured. Hematological and biochemical parameters were also measured. Serum cotinine, TOS, OSI, PCO, AOPP and LOOH levels were higher, whereas TAS and Cu, Zn-SOD levels were lower in ETS group. In ETS group INF-γ, IL-1β, IL-2, and IL-6 levels were higher. The Cu level was higher in ETS group. Blood reticulocyte number, serum creatinine and glucose were higher in ETS group. It could be concluded that exposure to tobacco smoke in cats impaired the oxidant/antioxidant balance and potentially triggered the release of pro-inflammatory cytokines.

Introduction

Exposure to passive smoking or environmental tobacco smoke has been associated with many risk factors for infants, children and non-smoker adults.[1] Household pets maintain close physical relationship with their owners. Cats are exposed to tobacco smoke directly by inhalation (second-hand smoke) and indirectly by ingesting smoke residues contaminated on skin, clothing and other surfaces (third-hand smoke).[2] Due to their frequent self-grooming habits, cats are at high risk of tobacco smoke exposure.[2] The harmful effects of environmental tobacco smoke on humans have been widely studied, however, only a few studies have been carried out on cats and dogs. Previous studies were mostly based on owner reported questionnaires, investigating the association between environmental tobacco smoke and the incidence of some diseases such as lymphoma and oral carcinoma.[3]

In humans, it has been reported that active and passive smoking cause oxidative stress which plays an important role in the pathogenesis of many diseases such as cancer, cardiovascular and respiratory diseases.[4] Oxidative stress is a situation in which there is an imbalance between the amount of reactive oxygen species (ROS) and antioxidant capacity in the body.[5] Tobacco smoke contains both gas and particular forms of free radicals and other oxidants which may enhance production of ROS in the body.[6] Excessive amounts of ROS damage all biological macromolecules which lead to impairment of normal physiological functions, thus, cause the development of many diseases.[6] As a result of oxidized macromolecules, oxidative damage products such as lipid hydroperoxide (LOOH), protein carbonyl (PCO), advanced oxidation protein products (AOPP) are formed and these are used as biomarkers to assess oxidative damage.[6] Enzymatic antioxidants such as copper, zinc superoxide dismutase (Cu, Zn-SOD), catalase (CAT) and non-enzymatic antioxidants

***Correspondence:**

Berjan Demirtas. DVM, PhD
Department of Plant and Animal Production, Equine and Training Program, Vocational School of Veterinary Medicine, Istanbul University-Cerrahpaşa, Avcilar, Istanbul, Türkiye
E-mail: berjan@iuc.edu.tr

such as thiol (T-SH) fractions scavenge excessively produced ROS and prevent the generation of oxidative stress.[7] Although specific biomarkers clearly identify oxidative stress, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) are used to define oxidant/antioxidant imbalances.[8,9] Tobacco smoke may enhance oxidative stress either increasing production of ROS or attenuating of antioxidant defense system in humans.[8] Tobacco which contains many trace elements, affects the concentration of trace elements in the body.[10] Trace elements play an important role in the formation of ROS and act as cofactors of antioxidant enzymes.[11] Tobacco smoke also affects a wide range of immunological functions in humans and laboratory animals.[12] Smoking is associated with both release and inhibition of pro-inflammatory cytokines.[13]

Nicotine and its major metabolites cotinine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) are used for determining the tobacco smoke exposure in pets.[2,3,14] Hair nicotine, urinary nicotine and NNAL/ cotinine levels have been used to detect environmental tobacco smoke in cats.[3,14]

There is no study about the effect of environmental tobacco smoke on oxidative stress, pro-inflammatory cytokines and trace elements in cats. Therefore, the objectives of this study were as follows: (1) to determine the serum cotinine levels and (2) to evaluate oxidative stress parameters, pro-inflammatory cytokines, some trace elements and blood parameters in healthy pet cats exposed to environmental tobacco smoke.

Materials and Methods

Subjects and study design. This project was granted by Research Fund of Istanbul University-Cerrahpaşa (No. TSA-2018-28834, 13.06.2018). The study was approved by the Animal Ethical Research Committee of Istanbul University- Cerrahpaşa (No. 2018/10, 02.03.2018) and conducted in the Animal Hospital of Veterinary Faculty between the years of 2018 and 2020. Cat owners who applied to the animal hospital for general examination and vaccination were informed about the study before their visit. On the day of their visit, cat owners signed the informed consents and filled a questionnaire about the general information of their cats (age, gender, breed, castrated/uncastrated, feeding type, vitamin/antioxidant supplementations and living environment) and the smoking habits of household members (number of cigarettes consumed by household members per day, duration of smoking, inside/outside the house). A total number of 74 cats underwent clinical examination and the questionnaire assessments. The cats with abnormal parameters in serum routine biochemistry, hematology or being positive for feline corona virus (FCoV), feline immunodeficiency (FIV), feline leukemia virus (FeLV) and toxoplasma tests were excluded from the study. Healthy cats were also excluded from the study where they had taken any medication and antioxidant/vitamin supplements in the last two months. Based on all history, clinical and laboratory examinations as well as owners' questionnaire reports, 40 healthy young adult indoor cats within the age of one to seven years were included in the study. Cats which were only indoor (*i.e.* not going outside) and fed on commercial cat food were included in the study. According to the owners' questionnaire, cats were divided into two groups: 1: Exposed to tobacco smoke (ETS; n = 20, female/ male = 13/7), 2: Non-exposed to tobacco smoke (NETS; n = 20, female/male = 14/6). In exposed group, the household members consumed > 10 cigarette per day inside their houses for at least one year, whereas, in non- exposed group households had no smoking history. Cats were of mixed-breed [both in ETS and NETS, n = 17 and pure-breed (both in ETS and NETS, n = 3 (in ETS: 2 Siamese and 1 Persian; in NETS: 1 Siamese, 1 British and 1 Persian]. The numbers of castrated/uncastrated cats were 14/6 in both ETS and NETS groups. Mean age was 4.00 ± 3.10 and 3.70 ± 2.10 years old for ETS and NETS, respectively. Gender distribution (male/female ratio) was similar in ETS and NETS groups. Fasting venous blood samples were collected in tubes containing ethylene-diaminetetraacetic acid (EDTA) for whole blood and plasma samples and in tubes without any anticoagulants for serum samples. To obtain serum and plasma, the tubes were centrifuged at 4,100 rpm for 5 min at 4.00 ˚C. The whole blood and serum samples were used immediately for the hematological and routine biochemical analyses, respectively. Remaining plasma and serum samples were aliquated and stored at – 80.00 ˚C until the analyses of serum cotinine, redox status biomarkers, trace elements and pro-inflammatory cytokines. Absorbance values of all redox status and pro-inflammatory cytokine biomarkers were recorded with optic reader (Synergy H1, Hybrid Multi-Mode Microplate Reader; BioTek, Winooski, USA). The concentrations were determined according to Beer-Lambert law. It describes how attenuation of light relates to the concentration of the medium through which it travels. The concentrations were calculated either with Lambert-Beer formula ($A = \varepsilon \times l \times c$) where, A means absorbance, ε is extinction coefficient, l is length of light that pass into solution and c; concentration or using corresponding standards.[15]

Cotinine analysis. Exposure to cigarette smoke was determined by cotinine analysis in cat serum. Cotinine levels of all samples were measured using a commercial ELISA kit specific for cat (Cat Cotinine; Sunred, Shanghai, China). The sensitivity of assay kit was 0.316 ng L^{-1}.

Measurement of TOS, TAS and OSI. Serum TOS and TAS levels of all samples were determined using commercial ELISA kits specific for cats (YL Biont, Shanghai, China). The sensitivities of assay kits were 0.222 pg mL^{-1} and 0.140 pg mL^{-1}, respectively. OSI was calculated

via the ratio of TOS: TAS as mentioned in the literature.[9]

Determination of oxidant status-related biomarkers. All highest analytical grade chemicals were purchased from Sigma Aldrich (St. Louis, USA). Concentrations of LOOH were estimated by the Wolff method.[16] Samples (50.00 µL) and ferrous oxidation with xylenol orange version 2 reagent (950 µL) were added to the tubes. After incubation of 30 min in the darkroom, the samples were centrifuged at 3,000 *g* for 10 min and the absorbance values of supernatants were recorded at 560 nm wavelength. Concentrations of PCO were analyzed as the previously defined colorimetric method.[17] Serum samples were reacted with 10.00 mM 2,4-dinitrophenylhydrazine reagent (1:4 ratio). After the precipitation process with cold trichloroacetic acid, the pellets underwent extensive washing with an organic solvent (ethanol: ethyl acetate 1:1 ratio) three times. Lastly, the resulting pellet was dissolved in guanidine-HCl (6.00 N) solution and the absorbance values of the final samples were measured at 360 nm. Levels of AOPP were analyzed by a previously defined method.[18] Samples (20.00 µL) were added to the plate. Two hundred microliter of citric acid were then added and mixed. Then potassium iodide was added. AOPP concentration was expressed as µmol L^{-1} of chloramine-T equivalents. The absorbance of test samples as well as chloramine-T standards was recorded at 340 nm.

Measurement of enzymatic and non-enzymatic antioxidant biomarkers. All highest chemicals were purchased from Sigma- Aldrich. Activity of Cu, Zn-SOD was analyzed using the previous method.[19] Enzyme activity was determined by measuring the inhibition rate of substrate hydrolysis in the assay mixture containing 0.30 mmol L^{-1} xanthine, 0.60 mmol L^{-1} Na_2EDTA, 150 µmol L^{-1} nitroblue tetrazolium, 400 mmol L^{-1} sodium carbonate and 1.00 g L^{-1} bovine serum albumin. The assay mixture was adjusted at pH 10.20 and xanthine oxidase (167 U L^{-1}) was added. After 20 min of incubation, 0.80 mmol L^{-1} copper (II) chloride was added to terminate reaction. The final absorbance values were recorded at 560 nm. Activity of CAT was determined by the method of measuring the rate of decomposition of hydrogen peroxide.[20] One catalase unit is the amount of catalase decomposing 1.00 µmol of hydrogen peroxide per min at pH 7.00 at 25.00 ˚C. The absorbance value was recorded at 240 nm. The T-SH of the plasma were assayed spectrophotometrically by Sedlak and Lindsay's method.[21] A volume of 20.00 µL of supernatant, 200 µL of Tris buffer (pH 8.20) and 10.00 µL of 5,5-dithiobis 2-nitrobenzoic acid were mixed to determine T-SH. The absorbance values were read at 412 nm wavelength.

Measurement of pro-inflammatory cytokines. The serum levels of IL-1β, IL-2, IL-6, IL-8, TNF-α and IFN-γ were determined using commercial ELISA kits specific for cats (all from YL Biont). The sensitivities of assay kits were 0.20 ng L^{-1}, 1.12 ng L^{-1}, 0.62 ng L^{-1}, 0.28 ng L^{-1}, 1.62 ng L^{-1} and 0.51 ng L^{-1}, respectively.

Measurement of Fe, Zn, Se, and Cu levels. Trace elements (Fe, Zn, Se and Cu) levels were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Nexion 1000; Perkin Elmer, Waltham, USA). Then, 0.10 - 0.50 g of each sample were dissolved in 5.00 mL of 65.00% nitric acid and 0.50 mL of peroxide at 200 ˚C in microwave. Next, ultra distilled water was added to solution to make up final volume of 35.00 mL. The standards of elements at 1.00, 5.00, 20.00 and 60.00 ppm were prepared and recorded with the blank solution. Calibration curves for all of four elements were obtained. Solutions were again diluted with ultra distilled water by taking 1.00 mL from the 35.00 mL solution and making it up to 5.00 mL. Dilution factors and weighing were recorded to ICP-MS system. The final concentrations were obtained from ICP-MS.

Statistical analysis. Statistical analyses were performed using the SPSS Software (version 25.0; IBM Corp., Armonk, USA). The normality of the distributed variables was assessed by Shapiro-Wilk test. The normal and non-normal distributed variables were respectively evaluated by independent samples *t*-test or Mann Whitney-U test. The results were expressed as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

Results

Serum cotinine levels and biomarkers of redox status are presented in Table 1. Cotinine level was significantly higher ($p < 0.001$) in ETS group than NETS group. In ETS group TOS and OSI values were significantly higher ($p = 0.033$ and $p = 0.001$, respectively), whereas, TAS value was significantly lower ($p = 0.001$). PCO, AOPP and LOOH levels were found significantly higher ($p < 0.001$, $p < 0.001$ and $p = 0.037$, respectively), whereas, Cu, Zn and SOD levels were significantly lower ($p = 0.004$) in ETS group than NETS group. There were not significant differences in CAT and T-TSH levels between two groups (both $p > 0.05$).

Serum trace element levels are shown in Table 2. Copper level was significantly higher ($p = 0.038$) in ETS group, whereas no significant differences were found in Fe, Zn and Se levels between two groups ($p > 0.05$ for all comparisons).

The cytokine levels are presented in Table 3. The levels of INF-γ, Il-1β, IL-2 and IL-6 were significantly higher ($p = 0.003$, $p = 0.002$, $p < 0.001$ and $p = 0.006$, respectively) in ETS group, whereas no significant differences were found in IL-8 and TNF-α levels between two groups ($p < 0.05$ for both comparisons).

The hematological and biochemical values are shown in Table 4. Blood reticulocyte number, serum creatinine and glucose were significantly higher ($p = 0.038$, $p = 0.023$ and $p = 0.03$, respectively) in ETS group, whereas no significant differences were observed in other hematological and biochemical parameters between two groups ($p > 0.05$).

Table 1. Cotinine levels and biomarkers of redox status in exposed to cigarette smoking (ETS) and non-exposed to cigarette smoking (NETS) groups. Data are presented as mean ± SD.

Parameters	ETS (n = 20)	NETS (n = 20)
Serum cotinine (ng mL^{-1})	2.23 ± 0.47	1.64 ± 0.59‡
TOS (ng L^{-1})	6.23 ± 1.91	4.89 ± 1.37*
TAS (ng L^{-1})	1.46 ± 0.54	2.15 ± 0.65‡
Oxidative stress index	5.39 ± 3.83	2.56 ± 1.27‡
PCO (nmol mg^{-1} protein)	1.68 ± 1.05	1.44 ± 0.10‡
AOPP (µmol L^{-1} chloramine-T eq.)	88.7 ± 5.00	81.2 ± 6.30‡
LOOHs (nmol mg^{-1} protein)	0.99 ± 0.11	0.91 ± 0.13*
T-SH (nmol mg^{-1} protein)	7.02 ± 1.13	8.01 ± 2.31
Cu, Zn-SOD (U mg^{-1} protein)	1.04 ± 0.42	1.46 ± 0.45†
Catalase (U mg^{-1} protein)	0.61 ± 0.06	0.62 ± 0.08

TOS: Total oxidant status; TAS: Total antioxidant status; PCO: Protein carbonyl groups; AOPP: Advanced oxidation protein products; LOOHs: Lipid hydroperoxides; T-SH: Total thiol fractions; Cu, Zn-SOD: Copper, zinc -superoxide dismutase activity.
*†‡ indicate significant differences at $p < 0.05$; $p < 0.01$; $p < 0.001$, respectively.

Table 2. Trace element levels (ppm) in exposed to cigarette smoking (ETS) and non-exposed to cigarette smoking (NETS) groups. Data are presented as mean ± SD.

Elements	ETS (n = 20)	NETS (n = 20)
Iron	3.94 ± 1.52	4.63 ± 2.73
Zinc	1.88 ± 1.30	2.75 ± 3.30
Copper	1.64 ± 0.59	1.42 ± 1.12*
Selenium	0.55 ± 0.08	0.51 ± 0.14

* indicates significant differences at $p < 0.05$.

Table 3. Cytokine levels in exposed to cigarette smoking (ETS) and non-exposed to cigarette smoking (NETS) groups. Data are presented as mean ± SD.

Parameters	ETS (n = 20)	NETS (n = 20)
IF-γ (ng L^{-1})	1.24 ± 0.50	0.81 ± 0.36*
TNF-α (ng L^{-1})	21.80 ± 10.70	26.10 ± 14.40
IL-1β (ng L^{-1})	5.48 ± 1.87	3.49 ± 1.93*
IL-6 (ng L^{-1})	6.83 ± 0.93	5.24 ± 2.16*
IL-8 (ng L^{-1})	31.10 ± 6.30	31.10 ± 5.80
IL-2 (ng L^{-1})	54.80 ± 7.10	42.80 ± 6.80†

IF-γ: Interferon gamma; TNF-α: Tumor necrosis factor alpha; IL: Interleukin.
*† indicate significant differences at $p < 0.01$; and $p < 0.001$, respectively.

Discussion

The effect of passive smoking on oxidative stress and pro-inflammatory cytokines in children and non-smoker adults has been widely studied. There is no study about the effect of tobacco smoke on oxidative stress and pro-inflammatory cytokines in pet animals. In the present study, we aimed to determine the tobacco smoke exposure using cotinine biomarker and measure the effects of environmental tobacco smoke on oxidative stress biomarkers, pro-inflammatory cytokines, some trace elements and blood parameters in healthy indoor cats.

Cotinine is one of the important biomarkers to determine tobacco smoke exposure. In our study, serum cotinine levels were found higher in ETS group. It has been shown that cats and dogs exposed to tobacco smoke have higher cotinine levels in their urinary samples compared to those inhabited in non-smoking enviroment.[14,22] Similarly, children receiving second-hand smoke due to parental smoking have higher serum cotinine levels compared to children living in non-smoker households.[23]

To our best knowledge, this was the first study to measure oxidative stress parameters in pet animals exposed to passive smoking. In our study, TOS and OSI values were found significantly higher, whereas TAS was significantly lower in ETS group. Our results showed that exposure to tobacco smoke was associated with an increase in total oxidants and a simultaneous decrease in total antioxidants and could cause oxidative stress**.** This was consistent with human studies regarding the development of oxidative stress induced by passive smoking. Infants and children exposed to parental passive smoking increased OSI as a result of increase in TOS and decrease in TAS values.[24] It has been also reported that maternal active and passive smoking causes oxidative stress in human cord blood and placental tissues.[25] Moreover, human neonates exposed to maternal smoking in utero showed impaired oxidant / antioxidant balance and developed oxidative stress.[26] Similar to human studies, in laboratory animals, rat offsprings which were maternally exposed to tobacco smoke showed lower TAS values and higher TOS values.[27]

In our study, LOOH, PCO and AOPP levels were higher in ETS group. This was the first study to measure the oxidation products in cats exposed to tobacco smoke. Similar to our results, LOOH levels were higher in mice exposed to passive cigarette smoking.[28] In humans, it has been reported that maternal passive smoking caused higher levels of LOOH in cord blood and fetuses.[29] The PCO levels were found higher in non-human primates exposed to perinatal passive smoking.[30] In another study, AOPP levels were higher in umbilical cord of human neonates exposed to maternal smoking.[31] Our results were consistent with the human and animal studies showing that the oxidation of lipids and proteins were occurred in cats as a result of tobacco smoke exposure.

To our best knowledge, this was the first study to measure enzymatic antioxidants in pet animals exposed to passive smoking. In the present study, we found that Cu, Zn and SOD levels were lower in ETS group, whereas there were no significant changes in CAT and T-SH values between the two groups. In laboratory animals, it has been reported that cigarette smoke reduces the serum SOD activity of healthy mice.[32] Similarly, it has been shown that human new-borns and children exposed to passive smoking have lower levels of Cu, Zn-SOD activity.[33] Similar to our results, it has been reported that CAT and T-SH

Table 4. Hemotological and Biochemical parameters in exposed to cigarette smoking (ETS) and non-exposed to cigarette smoking (NETS) groups. Data are presented as mean ± SD.

Parameters	ETS (n = 20)	NETS (n = 20)
RBC (× 10^6 µL^{-1})	10.30 ± 1.62	9.36 ± 1.94
Hematocrit (%)	44.45 ± 6.54	40.54 ± 8.74
HB (g dL^{-1})	13.61 ± 3.85	12.98 ± 2.68
MCV (fL)	43.41± 3.68	43.42 ± 4.36
MCH (pg)	13.92 ± 1.06	13.89 ± 0.89
MCHC (g dL^{-1})	32.12 ± 1.11	32.16 ± 2.16
RDW (%)	24.71 ± 2.93	24.49 ± 3.26
RET(× 10^3 µL^{-1})	28.67 ± 17.71	18.36 ± 11.69*
RET-HGB (pg)	15.97 ± 1.09	15.59 ± 1.45
WBC (× 10^3 µL^{-1})	8.11 ± 2.83	8.71 ± 3.37
Neutrophil (× 10^3 µL^{-1})	3.85 ± 1.69	4.51 ± 2.56
Lymphocyte (× 10^3 µL^{-1})	3.34 ± 1.30	3.30 ± 1.33
Neutrophil/Lymphocyte	1.21 ± 0.51	1.60 ± 1.31
Monocyte (× 10^3 µL^{-1})	0.26 ± 0.10	0.37 ± 0.21
Eosinophil (× 10^3 µL^{-1})	0.60 ± 0.34	0.48 ± 0.29
Basophil (× 10^3 µL^{-1})	0.05 ± 0.03	0.05 ± 0.03
Platelet (× 10^3 µL^{-1})	257.20 ± 57.30	231.10 ± 108.50
MPV (fL)	16.09 ± 1.25	16.81 ± 1.72
Plateletcrit (%)	0.41 ± 0.10	0.38 ± 0.17
Creatinine (mg dL^{-1})	1.47 ± 0.43	1.16 ± 0.39*
Glucose (mg dL^{-1})	113.00 ± 42.45	94.95 ± 15.01*
BUN (mg dL^{-1})	21.80 ± 3.37	20.25 ± 3.54
BUN/Creatinine	16.32 ± 6.31	20.28 ± 11.25
Total protein (g dL^{-1})	7.78 ± 0.68	7.45 ± 0.85
Albumin (g dL^{-1})	3.27 ± 0.23	3.32 ± 0.38
Globulin (g dL^{-1})	4.50 ± 0.67	4.15 ± 0.63
Albumin / Globulin	0.74 ± 0.13	0.81 ± 0.13
ALT (U L^{-1})	82.15 ± 42.48	100.75 ± 92.52
ALP (U L^{-1})	36.35 ± 19.90	50.95 ± 47.56

RBC: Red blood cell; HB: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red cell distribution width; RET: Reticulocyte; WBC: White blood cell; MPV: Mean platelet volume; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BUN: Blood urea nitrogen.
* indicates significant differences at $p < 0.05$.

levels are not significantly different between children exposed to passive smoking and those non-exposed.[34]

Tobacco smoke which contains many trace elements can alter the trace elements concentrations.[10] Trace elements such as Fe, Cu, Zn and Se act as cofactors of many antioxidant enzymes and also play important roles in the formation of ROS.[35] The present study was the first to measure serum trace elements in pet animals exposed to tobacco smoke. In our study, the Cu level was significantly higher in ETS group, whereas no significant changes were observed in Fe, Zn and Se levels between two groups. In laboratory animals, rats exposed to second-hand tobacco smoke had higher levels of Cu in kidney, however, lower in spleen tissues.[36] On the contarary, Fe levels were higher in spleen, however, lower in kidney tissues.[36] In children exposed to passive smoking, Cu and Fe levels have been found significantly higher.[37] Similarly, it has been reported that there is a significant increase in Cu level of children exposed to tobacco smoke, whereas there are not significant changes in Se levels between exposed and non-exposed groups.[38] In our study, the higher level of Cu might have showed that Cu acted as pro-oxidant and catalyze Fenton-like reactions which might have caused the accumulation of free radicals, thus, led to increase in TOS, OSI, LOOH, PCO and AOPP values.

In humans, active and passive smoking cause alterations in inflammatory cytokines.[39] Our study was the first to measure pro-inflammatory cytokines in cats exposed to tobacco smoke. We found that INF-γ, IL-1β, IL-2 and IL-6 levels were higher in ETS group, whereas there were no changes in TNF-α and IL-8 levels between the two groups. Tobacco smoke, which is the one of the causes of oxidative stress, induces inflammation and the release of pro-inflammatory cytokines.[39] In laboratory animals, rats exposed to passive smoking had higher levels of IL-2 and IL-6.[40] In another study, children exposed to passive smoking had lower levels of IFN-γ , IL-1β, IL-4 and IL-5 than those with no exposure.[41] It has been reported that smoking is associated with both release and inhibition of pro-inflammatory cytokines.[13] In our study, the higher levels of pro-inflammatory cytokines might cause the inflammation or the development of some allergic and autoimmune diseases in cats exposed to tobacco smoke.

In our study, although reticulocyte number was in reference interval, it was higher in ETS group. There were no significant differences in other hematological parameters including red blood cell, hemoglobin, hematocrit, white blood cell, and mean corpuscular volume counts. To our best knowledge, there is no study about measurement of hematological values in cats exposed to tobacco smoke. In laboratory animals, adult rat offsprings exposed to passive smoking had a higher number of total leukocytes and a lower mean corpuscular volume (MCV), whereas, no other erythrocyte parameters were changed.[42] In a human study, it was reported that erythrocyte indices were changed while total WBC was increased in passive smokers compared to non-smokers.[43] In another human study, it was reported that higher numbers of cigarette consumed was associated with increased number of reticulocytes.[44] Including different species or the amount and duration of smoking exposure might be one of the reasons of our different results in leucocyte numbers compared to those of laboratory animal and human studies. The higher number of reticulocytes in our ETS group might have been due to the compensation of hypoxia caused by carbon monoxide in tobacco smoke.

In the present study, ETS group showed higher levels of creatinine and glucose compared to NETS group. In humans, higher creatinine levels are associated with tobacco smoke.[45] It has been reported that kidneys might be damaged directly by toxins in tobacco smoke or in-directly by the oxidative stress induced by tobacco smoke.[46]

Cats with acute and chronic kidney diseases have higher levels of creatinine.[47] In our study, although creatinine levels were within the reference interval, it was higher in ETS group. Accordingly, higher levels of oxidative stress, pro-inflammatory cytokines and creatinine in ETS group indicated that cats exposed to tobacco smoke might be more predisposed to renal diseases. Human studies have shown that active and passive smoking are associated with impaired glucose tolerance and poor glycemic control.[48] Moreover, the incidence of type II diabetes mellitus was found to be higher in children and adults exposed to passive smoking.[49] In laboratory animals, it has been shown that rats exposed to passive smoking had higher glucose levels in their sera and urinary samples.[50] In our study, glucose levels were higher in ETS group although those of both groups were within the reference interval. Higher glucose levels with increased oxidative stress and pro-inflammatory cytokines might cause the pre-disposition to type II diabetes mellitus in pet cats exposed to tobacco smoke.

In conclusion, exposure to tobacco smoke in cats impairs the oxidant/antioxidant balance which might trigger the release of pro-inflammatory cytokines. Cu might act as a pro-oxidant and induce the production of free radicals. The increases in oxidative stress and pro-inflammatory cytokines as well as serum creatinine and glucose indicated that cats exposed to tobacco smoke might be predisposed to renal diseases and type II diabetes. Further studies are needed to find out association between passive smoking and feline diseases such as chronic renal diseases and type II diabetes.

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Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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