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Research Article

Effects of electronic cigarettes on oxidative stress markers in the rat kidney tissues

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Abstract

Objectives: Electronic cigarettes (e-cigarettes) are an alternative to traditional cigarettes. Although numerous studies have been conducted regarding the effects of traditional cigarettes on oxidative stress biomarkers in the kidney, there are only a few studies on the effects of e-cigarettes.

Methods: A total of 24 male Wistar albino rats were separated into three groups: Group 1 was treated with traditional cigarettes, Group 2 with e-cigarettes, and Group 3 formed the control group. Kidney homogenates and plasma samples were obtained, and the glutathione peroxidase, protein carbonyl, superoxide dismutase (SOD), catalase (CAT), lipid hydroperoxide (LPO), and symmetric dimethylarginine (SDMA) levels were examined.

Results: Higher plasma SDMA levels were determined in Group 1 and Group 2 compared with Group 3 (<0.0001). Higher SOD activity was found in Group 1 compared with Group 2 (p=0.0094). Lower CAT activity was found in Group 1 compared with both Group 2 (p=0.0035) and Group 3 (p<0.0001). Higher LPO levels were determined in the traditional cigarette smoking group compared with the control group (p=0.028), and no statistically significant difference was found between the e-cigarette and the control groups.

Conclusion: E-cigarettes and traditional cigarettes are associated with the dysregulation of particular oxidative stress markers in the kidney. However, e-cigarettes have less effect on some oxidative stress markers than traditional cigarettes. Long-term use of traditional cigarettes and e-cigarettes causes oxidative stress, which may lead to renal tissue damage and diminished kidney function.

Keywords: E-cigarette, kidney, oxidative stress, rat

The number of tobacco smokers worldwide increased to 1.1 billion in 2019. Tobacco smoke contains various compounds that are harmful to smokers and nonsmokers alike [1]. In 2013, the World Health Organization attributed 6 million deaths worldwide to smoking. Therefore, reducing smoking is a significant public health objective [2]. Most smokers want to quit smoking, but only about one out of every ten adult smokers succeeds [3]. Although traditional techniques to minimize smoking have been employed, such as organizing activities to highlight the dangers of smoking or boosting cigarette taxes, the number of smokers continues to increase. Therefore, there is a clear requirement for novel approaches in this respect [4, 5].

Electronic nicotine delivery devices, known as electronic cigarettes (e-cigarettes), have long been recognized as a safer alternative to traditional cigarettes. These battery-operated devices deliver nicotine to users via an aerosol carrier system, and their use is mainly prevalent among young people [6]. According to the American Center for Disease Control and Prevention, e-cigarette use among college students surged from 1.5% in 2011 to 20.8% in 2018 [7]. However, although it was initially thought that e-smoking reduced smoking and the associated health risks [8], it has more recently been associated with increased oxidative stress and inflammation-related vascular and cardiac dysfunction [7]. Furthermore, cytotoxicity, oxidative stress, airway hyperreactivity, mucin formation,

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apoptosis, and emphysematous alterations have also been linked to e-cigarette use in animal studies [9].

Free radicals such as the hydroxyl radical, hypochlorite, superoxide anion, and hydrogen peroxide are classified as reactive oxygen species. Free radicals are highly unstable molecules that cause cellular harm. Lipid peroxidation, DNA damage, and protein alteration are all caused by free radicals [10]. Various biomarkers, such as lipid hydroperoxide (LPO) and protein carbonyl (PCO), are used to assess the levels of oxidative stress [11]. There are different protection mechanisms in the body such as antioxidant enzymes to remove free radicals, the most widely known of which are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [12].

Although the effects of traditional cigarettes on the kidneys have been thoroughly researched, there are few studies on the impact of e-cigarettes on renal oxidative stress. The aim of this study was to compare the effects of e-cigarettes and traditional cigarettes on oxidative stress biomarkers in kidney tissue homogenates of male Wistar albino rats. This study can be considered to contribute to the literature to help clarify the molecular mechanism of e-cigarettes on kidney tissue.

Materials and Methods

Traditional cigarettes and e-cigarettes

Marlboro brand cigarettes containing tar, nicotine, and carbon monoxide (CO) smoke ranging from 7.4 to 10.3 mg tar/cigarette, 0.57 to 0.69 mg nicotine/cigarette, and 8.7 to 11.0 mg CO/cigarette were used for the cigarette exposure group. Joyetech eGo Aio 1500mAh was used as an e-cigarette device with a liquid mixture of 0.6 mg/mL nicotine in a 55/45 ratio of propylene glycol and vegetable glycerin, not containing any aromatizing or flavoring agents.

Exposure of the animals

A total of 24 adult male Wistar albino rats were randomly separated into 3 groups of 8, with traditional cigarette smoking and e-cigarette smoking applied to two groups and the third group forming a control group. All the rats in the cigarette and e-cigarette groups were exposed to 1.2 mg/h of nicotine. No intervention was made to the control group. At the end of the experiment, all the animals were euthanized. Detailed descriptions of the experimental procedures are given below. Approval for the study was granted by the Local Ethics Committee for Animal Research of Cumhuriyet University (Decision No.: 65202830-050.04.04-604).

All the rats were kept in standard laboratory conditions of a 12-h light/dark cycle (light between 06.00 a.m. and 6.00 p.m.) at a temperature of 22-24°C and 55% humidity. All procedures were carried out according to the guidelines outlined in the National Institute of Health's Guide to the Care and Use of Laboratory Animals.

Group 1 (n=7) (traditional cigarette group): The rats were exposed to 3 cigarettes in 1 h twice a day for 8 weeks, in specially designed glass bell jars. The CO and CO_2 levels were measured regularly during exposure. One rat in this group died during the experimental procedure and was not replaced.

Group 2 (n=8) (e-cigarette group): The rats consumed 2 mL of e-cigarette in 1 h twice a day for 8 weeks in specially designed glass bell jars.

Group 3 (n=8) (control group): No intervention was applied to this group during the 8 weeks.

End of the experiment

At the end of the experiment, all the rats were anesthetized with an intramuscular injection of 15 mg/kg xylazine and 90 mg/kg ketamine hydrochloride. Sodium pentobarbital was then administered intraperitoneally at a dose of 10 mg/100 g body weight for euthanasia. Bilateral kidneys were removed.

Preparation of tissue homogenates and isolation of subcellular organelles

All the procedures were performed as described by Sahoo [13]. The tissue was thawed, weighed, and then homogenized in ice-cold 10 mL/g phosphate saline buffer (0.01 M, pH 7.4). A quantity of 0.5 g tissue samples was weighed for analysis. The homogenates were then centrifuged for 5 min at 5000g, and the supernatant was kept at -80°C until analysis. The supernatant fluids were centrifuged at 10 000 rpm for 20 min at 4°C to separate the mitochondrial pellet, which was then washed three times with ice-cold phosphate buffer (0.01 M, pH 7.4) at 12 000 rpm for 5 min at 4°C. Biochemical analyses were performed immediately after isolation.

Blood samples

Intracardiac blood samples were obtained before euthanasia into lavender top tubes. The samples were immediately centrifuged for 10 min at 4000g and kept at -80°C until analyses.

Biochemical analyses

Quantitative ELISA kits were used to detect GPx (Cayman Chemical, Michigan 48108 USA), PCO (Sunred Bio, China), SOD (Cayman Chemical, Michigan, USA), CAT (Cayman Chemical, Michigan, USA), and LPO (MyBioSource, San Diego, USA) concentrations in the tissue samples. Plasma symmetric dimethylarginine (SDMA) levels were determined using the quantitative ELISA kit (Andy Gene, USA).

Statistical analyses

The data normality was evaluated with the Shapiro-Wilk normality test. Numerical variables were shown as either mean± standard deviation (SD) or median (1^{st} to 3^{rd} quartiles) values according to the distribution characteristics. The ANOVA test was used

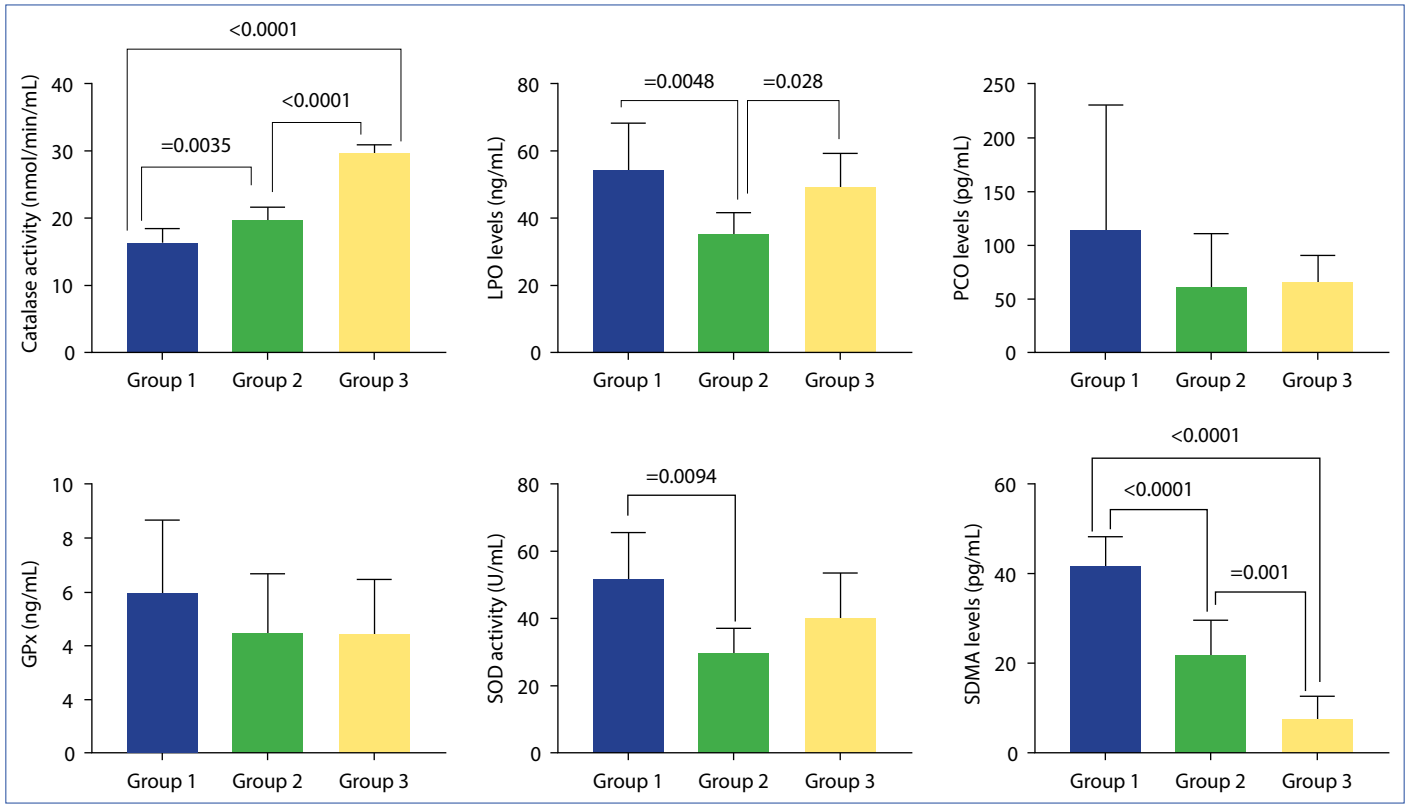

Figure 1. Box plots of GPx, PCO, SOD, CAT, and LPO.

GPx: Glutathione peroxidase; PCO: Protein carbonyl; SOD: Superoxide dismutase; CAT: Catalase; LPO: Lipid hydroperoxide.

to compare the glutathione GPx, SOD, CAT, LPO, and SDMA levels between groups, and Tukey's multiple comparisons test was used in all binary comparisons. The Kruskal-Wallis test was used to compare the PCO levels between groups, and Dunn's multiple comparisons test was used in binary comparisons. Analyses of the data obtained in the study were made using GraphPad Prism version 7.01 for Windows (GraphPad Software, La Jolla, CA, USA). A value of $p<0.05$ was considered statistically significant.

Results

Higher plasma SDMA levels were detected in the e-cigarette and traditional cigarette groups than in the control group. The SDMA levels were determined to be higher in the traditional cigarette group than in the e-cigarette group. The differences between the groups were statistically significant ($p<0.0001$) (Fig. 1). No statistically significant difference was determined between the groups in terms of GPx and PCO levels. Higher SOD activity was found in Group 1 compared with Group 2. Lower CAT activity was found in Group 1 compared with Group 2 and Group 3. The comparisons of the GPx, PCO, SOD, CAT, and LPO values are shown in Table 1 and Figure 1.

Discussion

The results of this study demonstrated higher plasma SDMA levels in the cigarette smoking and e-cigarette groups than in the control groups. SDMA can be formed by the enzymatic activity of arginine methyltransferases on L-arginine and asymmetric dimethylarginine (ADMA) residues [14]. In a previous study by Zhang et al., [15] higher levels of ADMA and SDMA and a lower level of L-arginine were reported to be associated with cigarette smoking. The current study finding in terms of SDMA levels was in accordance with that study. As the only chemical found in both groups was nicotine, this can be considered to be one of the main factors in the increasing concentration of SDMA levels in traditional cigarette and e-cigarette groups.

As SDMA has reverse effects on nitric oxide synthesis and a crucial role in endothelial dysfunction, it is accepted as an oxidative stress marker [15]. According to the current study results, both e-cigarettes and conventional cigarettes can be considered to increase the risk of endothelial dysfunction by increasing SDMA levels. However, lower SDMA levels were determined in the e-cigarette group than in the traditional cigarette smoking group; therefore, e-cigarettes may help reduce but not eliminate the endothelial dysfunction risk associated with higher SDMA levels.

One of the main molecular mechanisms of traditional cigarette smoking and e-cigarette is disrupted oxidative balance [16, 17]. SOD is responsible for the conversion of superoxide radicals to hydrogen peroxide. Therefore, SOD activity is an integral part of antioxidant capacity. Although previous studies have reported that traditional cigarette smoking decreased

Table 1. Comparisons of GPx, PCO, SOD, CAT, SDMA, and LPO values between groups

Parameters	Group 1 (n=7) (traditional cigarette)	Group 2 (n=8) (e-cigarettes)	Group 3 (n=8) (control)	p
GPx (ng/mL)	5.96±2.71	4.49±2.19	4.43±2.04	0.374
PCO (pg/mL)	69.27 (62.91-153.2)	55.41 (33.37-66.20)	67.23 (45.52-87.80)	0.227
SOD (U/mL)	51.68±13.54[a]	29.54±7.31[b]	39.78±13.44[b]	0.0127
CAT (nmol/mL/min)	16.44±1.44[a]	19.75±1.87[b]	29.73±1.21[c]	<0.0001
SDMA (pg/mL)	41.58±6.58	21.84±7.80	7.71±5.00	<0.0001
LPO (ng/mL)	54.29±13.85[a]	35.18±6.41[b]	49.50±9.71[a]	0.004

Results are given as mean±SD. Different superscripts in different rows indicate statistically significant difference between groups. P<0.05 was assumed statistically significant.
GPx: Glutathione peroxidase; PCO: Protein carbonyl; SOD: Superoxide dismutase; CAT: Catalase; SDMA: Symmetric dimethylarginine; LPO: Lipid hydroperoxide.

SOD levels [18-22], there are also contradictory findings [23]. In the current study, higher SOD activity was determined in the cigarette smoking group than in the control and e-cigarette groups. The contradiction between studies may be related to differences in the studied samples, the duration of exposure to cigarette smoke, and the SOD determination method. A previous study showed that SOD prevented smoke-induced inflammation and proteolytic cascade that led to emphysema formation in two separate animal models [24]. Accordingly, it can be thought that increased SOD activity has a role in eliminating the increased superoxide radicals and preventing traditional cigarette smoke-induced oxidative stress in the kidney.

The current study results showed no statistically significant difference between the e-cigarette and control groups regarding SOD activity in the kidney tissue homogenates. Taylor et al. [16] showed that e-cigarettes cause lower oxidative stress than traditional cigarette smoking. Accordingly, it can be considered that e-cigarettes produce fewer superoxide radicals, resulting in lower SOD activity when compared with the traditional cigarette smoking group.

CAT is an endogen enzyme responsible for converting hydrogen peroxide to water and oxygen. In the current study, decreased CAT activity in the traditional cigarette smoking group compared with the control and e-cigarette groups was observed. Previous studies have reported that the use of traditional cigarettes is associated with decreased CAT activity [25, 26]. The current study finding is consistent with those earlier studies. In the current study, lower CAT activity was observed in the e-cigarette group compared with the control group. Thus, it was concluded that e-cigarettes have less effect than traditional cigarettes in terms of CAT activity in the kidney tissue. However, in the light of these findings, it can be said that e-cigarettes and traditional cigarettes have a detrimental effect on CAT activity, as both cause increased oxidative stress by inhibiting antioxidative capacity in the kidney.

The oxidation of cell membrane lipids containing double carbon bonds by free radicals is known as lipid peroxidation [27]. In the current study, the levels of LPO, which are the intermediate lipid peroxidation products, were determined to be higher in the traditional cigarette smoking group compared with the control group. However, no statistically significant differences were determined between the e-cigarette and control groups. It is well known that traditional cigarette smoking is associated with *in vivo* and *in vitro* lipid peroxidation [28], and there has been less oxidative stress in e-cigarette groups than in traditional cigarette smoking groups [29]. Therefore, the current study findings are compatible with the literature. Elevated oxidative stress has been associated with acute and chronic kidney diseases [30]; therefore, as traditional cigarette smoking is thought to induce oxidative stress and inflammation in renal tissue, it may consequently promote kidney tissue injury.

In conclusion, both e-cigarette and traditional cigarette smoking are associated with dysregulation of the antioxidant enzyme balance and lipid peroxidation in the kidney tissue. However, this effect is lesser in e-cigarettes than in traditional cigarettes, which may be related to the much lower chemical content of e-cigarettes than conventional cigarettes. Oxidative stress is related to increased inflammation, so it can be thought that long-term e-cigarette and traditional cigarette use may cause chronic inflammation resulting in damage to kidney tissue and functions.

Conflict of Interest: The authors declare that there is no conflict of interest.

Ethics Committee Approval: The study was approved by The Sivas Cumhuriyet University Animal Research Local Ethics Committee (No: 65202830-050.04.04-604, Date: 30/11/2021).

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