The Effects of Melatonin and Endothelin-A Receptor Antagonist BQ-123 in Exposed to Smoke in Rat Renal Tissue

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ABSTRACT

It has been known that smoking increases the level of Endothelin (ET), a very powerful vasoconstrictor agent in circulation, causes damage and functional impairment of tissues. In our study, it was aimed to evaluate the effects of melatonin, a potent antioxidant, and BQ123 on renal tissue of rats exposed to cigarette smoke. Wistar albino 34 adult male rats were divided into four groups; Control, Smoking, Smoking+Melatonin, and Smoking+BQ-123 groups. They were exposed to cigarette smoke in the vacuum-operated glass cabinet 3 times a day, 30 minutes for 28 days. Melatonin was administered i.p. 25 mg/kg/every day. BQ-123 was injected from tail vein i.v. 1 mg/kg, on the first, 7th, 14th, 21th, 27th day. After the rats were sacrificed on the 28th day, the biochemical parameters were analyzed with spectrophotometric methods and tubular cell accounts for stereological analysis were done by optical fractionator technique. Renal tissue SOD activity was significantly increased in the Smoking+Melatonin group according to the control and Smoking+BQ-123 groups. Catalase activity was significantly decreased in the Smoking+Melatonin according to the other groups. Lipid peroxidation level was significantly increased in Smoking group in comparison with the Control and Smoking+BQ-123 groups. Protein Carbonyl and Nitric Oxide levels, and GSH-Px activity have not statically significant among the groups. Tubular cell accounts were significantly increased in the given of Melatonin and BQ-123 groups according to the Control and Smoking groups. We concluded that cellular damage generated by smoke in renal tissue of rats partially reduced by Melatonin and BQ-123 depending on the dose and duration with different pathways. This conclusion was supported by histologic staining and stereologic analysis with increase of the cell number of renal tissue in rats.

Key Words: *Smoking, Melatonin, BQ-123, Endothelin receptor antagonist*

Sigaraya Maruz Kalmış Sıçan Böbrek Dokusunda Melatonin ve Endotelin Reseptör Antagonisti BQ-123'ün Etkisi

ÖZET

Sigaranın dolaşımda çok güçlü bir vazokonstriktör ajan olan endotelin (ET) düzeyini artırdığı, doku hasarına ve fonksiyonunda bozulmaya yol açtığı bilinmektedir. Çalışmamızda, sigaraya maruz bırakılan sıçanların böbrek dokusunda, güçlü antioksidan melatonin ve ET reseptör antagonisti BQ-123'ün etkilerinin değerlendirilmesi amaçlandı. Erişkin erkek 34 Wistar Albino sıçan Kontrol, Sigara, Sigara+Melatonin, Sigara+BQ-123 olmak üzere 4 gruba ayrıldı. Günde 3 kez, 30 dakika, 28 gün süreyle cam kabin içinde vakumlayarak si-

garaya maruz bırakıldılar. Melatonin her gün 25 mg/kg dozda i.p., BQ-123 ise 0, 7, 14, 21, 27. günlerde kuyruk veninden 1 mg/kg dozunda verildi. Biyokimyasal parametreler spektrofotometrik yöntemle, tübüler hücre sayımları stereolojik metotlardan optik fraksiyonlama yöntemiyle değerlendirilmek üzere 28. günde sıçanlar sakrifiye edildi. Renal doku süperoksit dismutaz (SOD) aktivitesi Sigara+Melatonin grubunda, Kontrol ve Sigara+BQ-123 gruplarına göre anlamlı olarak arttı. Katalaz aktivitesi ise Kontrol, Sigara, Sigara+BQ-123 gruplarına göre Sigara+Melatonin grubunda anlamlı olarak azaldığı görüldü. Lipit peroksidasyon düzeyi, Sigara grubunda Kontrol ve Sigara+BQ-123 grubuna göre anlamlı olarak arttığı bulundu. Protein karbonil ve nitrik oksit düzeyleri ile glutatyon peroksidaz (GSH-Px) aktivitesinde istatistiksel olarak anlamlı bir farklılık görülmedi. Tübüler hücre sayıları BQ-123 ve Melatonin verilen gruplarda Kontrol ve Sigara gruplarına göre anlamlı olarak arttığı görüldü. Sigaranın böbrek dokusunda oluşturduğu hücresel hasarı, Melatonin ve BQ-123'ün değişik yolaklarla doz ve süreye bağlı azalttığı düşünüldü.

Anahtar kelimeler: *Sigara içme, Melatonin, BQ-123, Endotelin reseptör antagonisti*

INTRODUCTION

Smoking which is a common habit among people are known to increase a lot of diseases related to respiratory system, testis, brain, heart, blood vessels, skin, kidney. Due to the risk of becoming and causing diseases, smoking leads to important health problems. It is located in the etiology of many diseases, for example, the inflammation of stomach and duodenum structures, chronic obstructive pulmonary disease (COPD), atherosclerosis, cancers, peptic ulcer disease, renal cell carcinoma (1).

Melatonin is endogenously secreted by the pineal gland, well-known in the role of circadian rhythm, having a very strong antioxidant activity. Receptor mediated effects stimulates the expression of intracellular antioxidant and detoxification genes via signal transduction (2). Melatonin has also strong reactive oxygen species (ROS) scavenging effect. Thus, it has a powerful protective effect against free radical damage cells and tissues (2,3). Direct and indirect effects of cigarette smoke on important oxidative evidence were obtained in scientific studies (4,5). Research was presented that people who cigarette smoke have either increase (6) or decrease (5) in circulating melatonin levels.

Endothelin (ET) a potent vasoconstrictor and mitogen agent involved in the regulation of vascular tone (7,8). The main type of ET is Endothelin-1 (ET-1) in human plasma (9,10). Discovered by Yanagisawa (11), ET has two types receptor in cell membranes. ETA receptors (ETAR) are primarily found on the vascular smooth muscle cell membrane. ET-1 via ETAR leads to vasoconstriction, mitogenesis, and anti-apoptotic effect with increased intracellular Ca+2 concentrations. ETB receptors (ETBR) are mainly found on the endothelial cell membrane. ET-1 via ETBR causes ET-1 clearance, cell death and vasodilation with nitric oxide (NO) and prostosiclin (PGI2) secretion (7,10,12). BQ-123 was firstly synthesis as ETAR antagonist (13), has antioxidant effects, and has protective effect from renal ischemia and reperfusion (14). ET-1 increases within ten minute following cigarette smoking in plasma (15, 16). It might be associated with the harmful effects of smoking such as atherosclerosis (15), essential hypertension (17), cardiovascular pathophysiology (18), renal dysfunction (19).

In that case, the damage of smoking might be improved by the treatment of Melatonin and ETAR antagonist. Our scans of the literature, the effects of melatonin and ET receptor antagonists against damage caused by cigarette smoking were not investigated in especially renal tissue. Therefore, we aimed of the current study was to determine that the rats exposed to cigarette smoke in renal tissue of rats were comparatively assessed the effects of melatonin as one of the most powerful antioxidant substances and BQ-123 as an ETAR antagonist.

MATERIAL and METHODS

Animals and animal treatments

Thirty-four Wistar albino adult male rats weighing 220-300g were used in the experimentations. The experiments were performed in accordance with "The National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) (Revised 1996)." The experiments were approved by the local ethical committee of the Medical School of Gaziosmanpasa University. All experiments were carried out in the laboratories at Gaziosmanpasa University. The rats were randomly assigned to three groups: Control group (n=10), Smoking group (n=9), Smoking+Melatonin group (n=5), and Smoking+BQ-123 group (n=10). They were exposed to smoking in the vacuum-operated glass cabinet (0.5x0.5x1m) 3 times a day, 30 minutes for 28 days. Rats exposed to three cigarette smoke in thirty minutes. Melatonin was administered i.p. 25 mg/kg/day. BQ-123 was injected from tail vein i.v. 1 mg/kg, on the first, 7th, 14th, 21th, 27th day. Rats were sacrificed on the 28th day under i.p. 30 mg/kg ketamine and 5

mg/kg xylazine anesthesia. Right kidney was removed with surgical technique for the analysis of biochemical parameters by spectrophotometric method, stored at −80 0C until enzymatic analysis. Homogenate, supernatant, and extracted samples (20) from the kidney were prepared for the antioxidant enzyme analysis of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and the levels of nitric oxide (NO), thiobarbituric acid reactive substance (TBARS), protein carbonyl (PC), protein (with Lowry's method) (21).

Renal Tissue Nitric Oxide (NO) Levels

The index parameters of NO, nitrite and nitrate levels, were measured by Griess reaction (22). The spectrophotometric analysis of NO was calculated by a standard curve, prepared with a set of serial dilutions of sodium nitrite.

Renal Tissue Superoxide Dismutase (SOD) Activity

The reduction of nitroblue tetrazolium was used for analysis of SOD activity (EC 1.15.1.1) (23). The SOD activity was measured from ethanol phase of extracted samples with ethanol/chloroform mixture (5/3, v/v). The results were given as unit that was defined as the amount of enzyme for 50% inhibition of nitroblue tetrazolium reduction rate.

Renal Tissue GSH-Px activity

The GSH-Px activity (EC 1.6.4.2) was analyzed according to alteration of H2O2 within a mixture of NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase (24). The activity was measured at 340 nm by a spectrophotometer.

Renal Tissue Thiobarbituric Acid Reactive Substances (TBARS) Level

The reaction of thiobarbituric acid with malondialdehyde or malondialdehyde like sbustances is used as index of lipid peroxidation (25). The reaction was done at 90-100 oC and TBARS were measured at 532 nm. We used a standard curve prepared from the experiments of 1,1,3,3-tetramethoxypropane solution with different concentrations in order to calculate the TBARS levels.

Renal Tissue Protein Carbonyl (PC) Content

The protein oxidation was analyzed by measurement of protein carbonyl (PC). The reaction of carbonyl groups with 2,4-dinitropheniylhydrazine causes the formation of 2,4-dinitropheniylhydrazone. We analyzed the tissue PC level at 360 nm spectrophotometrically (26).

Histological Examination

Left kidney was removed for the stereological analysis by optical fractionator technique, immersed in 10 % neutral formalin and post-fixed for 1 week at 4°C. Renal tissues were washed under running water for 12 hours. After washing, the tissues were embedded in paraffin applied to routine histological procedures. Tissues after it is embedded Rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany) with a 30 mm thick, systematic random style, thick sections were sampled the rate of 1/12. Additionally, the 5 µm thin sections were stained for Periodic Acid Schiff (PAS) stain for histological imaging and composition assessment. A stereology workstation for stereological analysis by optical fractionator technique was used. An unbiased estimation

Table 1*.Effects of Melatonin and BQ-123 to renal tubular cell number in the exposed to smoke in rats. Tubular cell numbers were significantly increased in the given of Melatonin and BQ-123 groups according to the Control and Smoking groups. Total volumes significantly increased in Control group according to the other groups.*

	Tubular Cell Number	Cortical Volume	Medullar Volume	Total Volume
1- Control	7,920.000±206.787	20,096.000±600.388	5,428.400±195.521	25,524.400±763.125
2- Smoking	8,088.720±245.245	9,360.000±294.381	2,804.000±147.160	12,164.000±302.218
3-Smoking+Melatonin	9,359.520±255.512	14,664.000±456.164	4,082.000±130.591	18,746.000±511.797
4- Smoking+BQ-123	9,409.400±213.495	15,782.000±553.402	4,950.000±231.582	20,732.000±650.426
P				
1*2	A.D.	0.001	0.001	0.001
1*3	0.001	0.001	0.001	0.001
1*4	0.001	0.001	A.D.	0.001
2*3	0.007	A.D.	0.001	0.001
2*4	0.004	0.001	0.001	0.001
3*4	A.D.	A.D.	0.017	A.D.

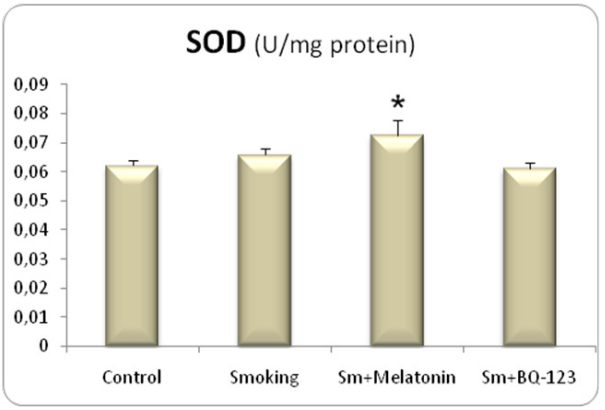

***Figure 1**. Effects of Melatonin and BQ-123 to renal tissue SOD (U/mg protein) activity in the exposed to smoke in rats. Renal tissue SOD activity was significantly increased in the Smoking+Melatonin according to the control (p<0.014) and Smoking+BQ-123 (p<0.008) groups.*

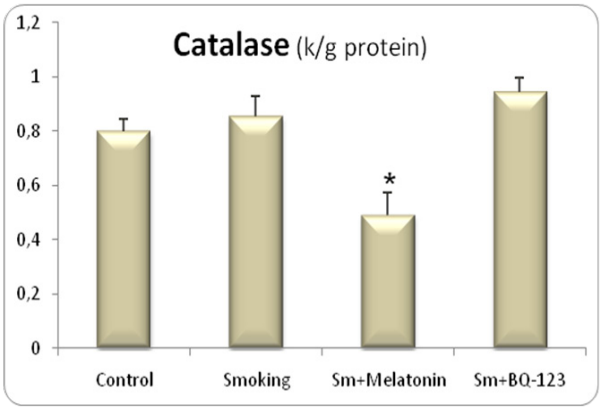

***Figure 2**. Effects of Melatonin and BQ-123 to renal tissue Catalase (k/g protein) activity in the exposed to smoke in rats. Catalase activity was significantly decreased in the Smoking+Melatonin according to Control (p<0.002), Smoking (p<0.001), Smoking+BQ-123 (p<0.001).*

of the total number of tubular cells from the kidney was obtained by choosing every 12th section, in accordance with the systematic random sampling procedure (27). The stereologic workstation consists of a trinocular microscope (Leica DM 2500; Leica Instruments, Nussloch, Germany), a motorized microscope stage (BioPoint 2; Ludl Electronics, Hawthorne, NY, USA), a digital microcator (Heidenhain; Traunreut, Germany), a color video camera (Q-imaging; Surrey, BC Canada), and personal computer and stereology software (Stereo Investigator; MBF Biosciences, Williston, VT, USA). Tubular cell numbers were estimated and counted according to the rules of unbiased counting (28-30). Cavalieri's principle was used calculating for total renal cortical and medullar volume (31).

Statistical Analysis

The one-sample Kolmogorov–Smirnov test was used for analyzing the groups' distribution. The one-way ANOVA test was performed and post hoc multiple compari-

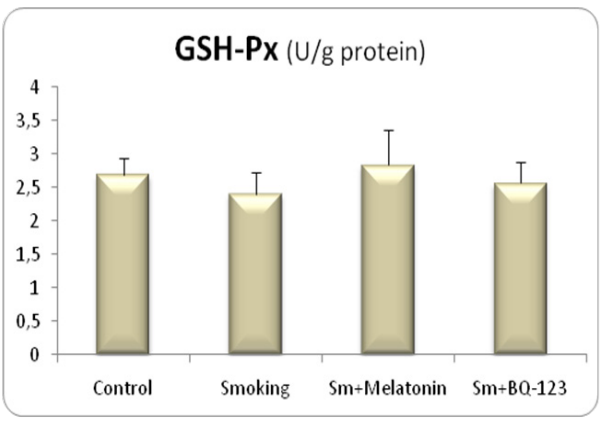

***Figure 3**. Effects of Melatonin and BQ-123 to renal tissue GSH-Px (U/g protein) activity in the exposed to smoke in rats. GSH-Px activity has not statically significant difference among the groups.*

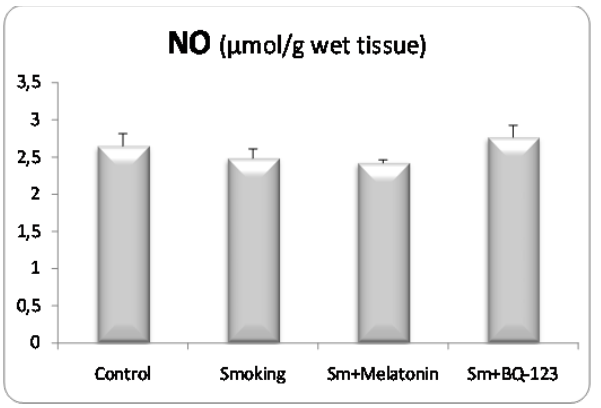

***Figure 4**. Effects of Melatonin and BQ-123 to renal tissue NO (µmol/g wet tissue) levels in the exposed to smoke in rats. NO levels have not statically significant difference among the groups.*

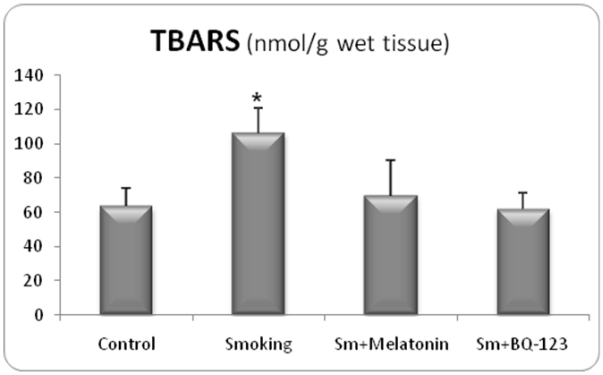

***Figure 5**. Effects of Melatonin and BQ-123 to renal tissue TBARS (nmol/g wet tissue) levels in the exposed to smoke in rats. TBARS level was significantly increased Smoking group according to the Control (p<0.014) and Smoking+BQ-123 (p<0.011) group.*

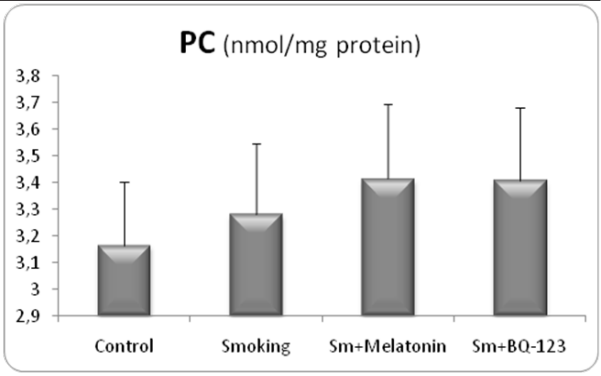

***Figure 6**. Effects of Melatonin and BQ-123 to renal tissue Protein Carbonyl (PC) (nmol/mg protein) levels in the exposed to smoke in rats. PC levels have not statically significant difference among the groups.*

sons were done with LSD for the normally distributed groups' variables. All data were analyzed using SPSS for Windows. Results are given as the means ± standard error of the mean. Level of significance is accepted as p-value <0.05.

RESULTS

Renal tissue SOD activity was significantly increased in the Smoking+Melatonin according to the control and Smoking+BQ-123 groups (Figure 1). Catalase activity was significantly decreased in the Smoking+Melatonin according to Control (p<0.002), Smoking (p<0.001), Smoking+BQ-123 (p<0.001) (Figure 2). GSH-Px activity has not statically significant difference among the groups, although non-significant an increase in Smoking+Melatonin group (Figure 3). Nitric Oxide levels have not statically significantly different among the groups (Figure 4). TBARS level was significantly increased in Smoking group according to the Control (p<0.014) and Smoking+BQ-123 (p<0.011) group, TBARS level of Smoking group has also non-significant an increase according to Smoking+Melatonin group (Figure 5). Protein Carbonyl levels have not statically significantly different among the groups (Figure 6). It was shown that effects of Melatonin and BQ-123 to renal tubular cell number in the exposed to smoke in rats (Table 1). Renal tubular cell numbers were significantly increased in the given of Melatonin and BQ-123 groups according to the Control

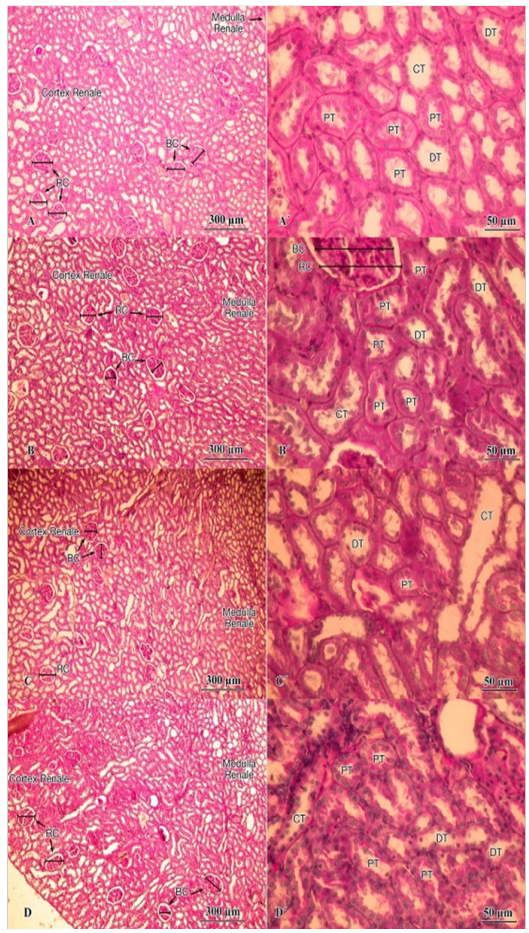

***Figure 7**. Representative photomicrographs of 5µ thin renal section with PAS stain. Renal tissue A: Control X4; A': Control X20; B: Smoking X4; B': Smoking X20; C: Smoking+Melatonin X4; C': Smoking+Melatonin X20; D: Smoking+BQ-123 X4; D': Smoking+BQ-123 X20.*

($p<0.001$) and Smoking ($p<0.007$ and $p<0.004$) groups. Total volumes significantly decreased in other groups according to the Control groups (Table 1). Besides, total volumes significantly increased Smoking+Melatonin ($p<0.001$) and Smoking+BQ-123 ($p<0.001$) groups according to the Control groups (Table 1).

As histo-pathologically, Smoking group according to Control group has observed tubular destruction, cellular cytoplasmic degeneration, and diminished apical membrane microvillus stain with PAS. We observed slight diminishing in the tubular damage in Smoking+Melatonin and Smoking+BQ-123 groups. This diminishing was noticeable in Smoking+BQ-123 group according to Smoking+Melatonin group.

DISCUSSION

We investigated to the exposed to cigarette smoked rats inquire with regard to the effect of melatonin and ETAR antagonist. We used biochemical analysis in terms of oxidative stress, as well as stereological methods. We found that Melatonin and BQ-123 in the kidneys of rats exposed to smoking cause an increase in the number of the cell with cell proliferation, effects of antioxidant systems in different ways at the end of the 28.day. During smoking, a large number of free radicals and reactive oxygen species are produced (4,5,32). Free radicals to proteins, carbohydrates, lipids, and DNA in cells on are caused oxidative damage (32). Lipid peroxidation was increased by smoking and was decreased by BQ-123 and Melatonin. However, Melatonin decreased its as statistically non-significant (Figure 5). In studies conducted by Ozan et al and Ramesh et al., it was also determined increasing TBARS level with smoking (33,34). The results of antioxidant enzyme activity are probably different depending on the dose and duration of treatment. Renal histologic assessment also shown has a little more recovery with BQ-123. The treatment of Melatonin caused to increase SOD activity and decrease CAT activity even after 28 day is noteworthy. Some researchers found that oral nicotine (100 µg/ml/day) was administered for 28 days increases ET-1 levels, and decreases SOD activity and NO level in endothelial cells. Melatonin (5 mg/kg/day) bring about decrease ET-1, increase SOD activity and NO level. They suggest Melatonin as a therapeutic intervention for smokers (35). We also found increase SOD activity after use cigarette smoke for 28 day in rats. However we administered i.p. melatonin 25 mg/kg/day. Smoking makes with sympathetic activation to the acute effects on the kidneys, causing long-term damage indicates that the vascular endothelial cell damage (36, 37). Smoking enhances the level of plasma ET-1 (15,16). It was informed that increased ET-1 level is associated with the harmful effects of smoking such as atherosclerosis (15), essential hypertension (17), cardiovascular pathophysiology (18), renal dysfunction (19). In the chronic renal failure and chronic hemodialysis patient was rise ET-1 level (38,39). There are positive correlation with serum creatinine and ET-1 (19). In addition, the increased level of plasma ET-1 causes the reduction in glomerular filtration rate, urine production renal blood flow (40-42). Therefore, we used ETAR antagonist for inhibiting the harmful effect of cigarette smoking in renal tissue for the first time. Our findings reinforced that ETAR antagonist in smokers might be recovery for the detrimental effects. We thought that the number of renal cell and volume of renal tissue might be increased with response to cellular damage depending on smoking. Melatonin and BQ-123 are known have antioxidant activity should be provoked this response (Table 1). Stereologic analysis is important for submitting a more reliable evaluation because of all tissue sampling. We firstly demonstrated that Melatonin and BQ-123 increase cell number and volume in renal tissue against cellular damage on smoking. In conclusion, the present study demonstrated that cellular depredation of smoke in renal tissue slightly reduced by Melatonin and BQ-123 depending on the dose and duration. These agents provoked increase of the cell number of renal tissue in rats. This result was promoted by stereologic analysis with increase of the cell number of renal tissue in rats and histologic staining. Smoking is one of the substantially continuing health problems worldwide. Our findings about orally Melatonin and ETAR antagonist could provide the basis for a therapeutic approach of improving smoking-induced renal damage. These are needed the use of convenient dose and duration.

Conflict of Interest Statement

The authors declare that there is no conflict of interests.

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