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Original article

PROTECTIVE EFFECT OF CELECOXIB ON NICOTINE TOXICITY AND ALTERATIONS IN TRACE ELEMENT LEVELS

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Abstract

The aim of this study was to investigate alterations in trace elements such as Copper (Cu) and Zinc (Zn) levels in rat tissues, body weights and liver weights dependent on nicotine exposure, and discuss the protective effect of celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor. We injected to rats at 1.5 mg/kg (ip) dose nicotine for 7 days period and observed effect of nicotine on trace element levels, body weights and liver weights of rats. Nicotine treatment reduced body weights. Alterations on body weight may be a consequence of reduced food intake and an increase in the amount of energy consumption due to metabolic changes. In addition we found that weights of the liver in the nicotine group were significantly lower than that in the control group. Nicotine treatment increased copper and zinc levels in all tissues. Increases of tissue Cu and Zn levels and serum Zn levels may depend on stimulation of defence system against nicotine exposure.

Key words: *Body weights, Nicotine, Oxidative stress, Trace elements.*

Nikotin Toksisitesi ve Eser Element Düzeylerindeki Değişiklikler Üzerinde Selekoksibin Koruyucu Etkisi

Bu çalışmanın amacı, nikotin maruziyetine bağlı olarak sıçan dokularında bakır (Cu) ve çinko (Zn) gibi eser element düzeyleri ile vücut ve karaciğer ağırlıklarındaki değişimleri incelemek ve bir seçici siklooksijenaz-2 (COX-2) inhibitörü olan selekoksibin koruyucu etkisini tartışmaktır. Çalışmamızda, sıçanlara 7 gün süreyle 1.5 mg/kg (ip) dozda nikotin enjekte ettik ve nikotinin sıçanlarda eser element düzeyleri, vücut ağırlıkları ve karaciğer ağırlıkları üzerindeki etkisini gözlemledik. Nikotin uygulaması vücut ağırlıklarını azalttı. Vücut ağırlıklarındaki değişim gıda alımının azalması ve metabolik değişimlere bağlı olarak enerji tüketiminin artışının sonucu olabilir. Ayrıca, nikotin grubunun karaciğer ağırlıklarını kontrol grubuna göre daha düşük bulduk. Nikotin uygulaması tüm dokularda bakır ve çinko düzeylerini arttırdı. Dokularda Cu ve Zn düzeylerindeki ve serum Zn düzeylerindeki artış nikotin maruziyetine karşı savunma sisteminin uyarılmasına bağlı olabilir.

Anahtar kelimeler: *Vücut ağırlıkları, Nikotin, Oksidatif stres, Eser elementler.*

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INTRODUCTION

Oxidative stress may cause severe tissue damage including lipid peroxidation (LPO), enzyme inactivation and DNA damage (1-3). Nevertheless, aerobic organisms are protected against oxidative stress by enzymatic and non-enzymatic antioxidant defenses. The defense system includes ascorbic acid (vitamin C), α-tocopherol (vitamin E), vitamin A and trace elements such as zinc (Zn^{+2}), copper (Cu^{+2}) and magnesium (Mg^{+2}) (4). Cyclooxygenase-2 (COX-2) is up-regulated in inflammation and cancer tissue (5). It has been shown that cancer development can inhibited in COX-2 knockout mice (6).

It is well recognized that essential trace elements have many biological functions in living organisms. The proposed mechanisms involve the antioxidant potential of trace elements such as Zn^{+2} and Cu^{+2} dependent enzyme system, induction of metallothionein, effects on immune response and DNA repair system. Trace elements are required in low concentrations for example as essential components of antioxidative enzymes. The cytoplasmic Cu/Zn-superoxide dismutase contains Zn^{+2} and Cu^{+2} as co-factors. One of the most significant functions of zinc is related to its antioxidant role and its participation in the antioxidant defense system (4, 7, 8).

Previous studies have shown that nicotine induces oxidative stress in various tissues (9,10). Nicotine and presumably therefore, smoking, depress the antioxidant system, in tissues such as liver, leading to elevated Zn^{+2} and Cu^{+2} levels causing the oxidative stress (10). Both altered trace element metabolism and cigarette smoking have been proposed to be risk factors for several diseases. Thus, it is important to identify the mechanisms by which nicotine and cigarette smoke alter trace element metabolism.

The aim of this study was to investigate the Zn^{+2} and Cu^{+2} levels in liver, lung, kidney, brain and heart tissues, in addition to body and liver weights. Effect of celecoxib, a selective COX-2 inhibitor, on nicotine-induced cellular injury were also evaluated to explain the role of COX-2 pathway.

EXPERIMENTAL

Animals

18 Wistar Albino male rats (250-300 g, n=5-7) were used to perform the study. The rats were divided into three groups:

Group 1 Control; i.p., saline (vehicle).

Group 2 Nicotine; 1,5 mg/kg i.p., nicotine.

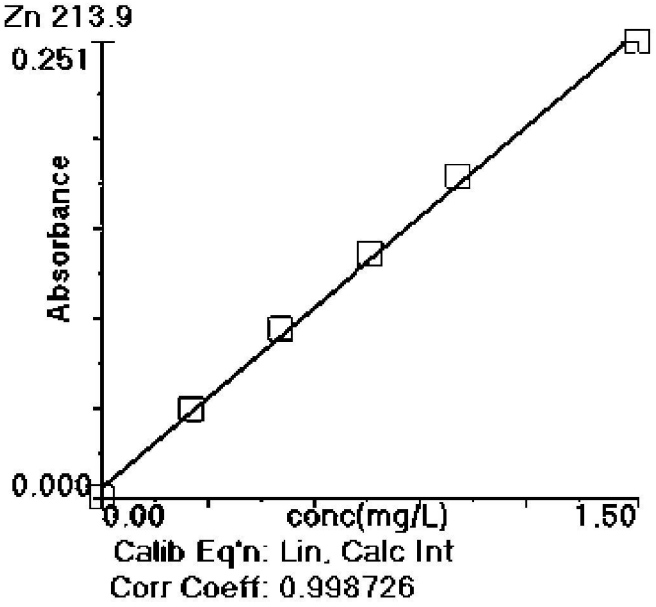
Group 3 Nicotine+Celecoxib; an hour ago 1,5 mg/kg i.p. nicotine injection, 15 mg/kg, i.p. celecoxib injected and provided standard pellet diet and water *ad libitum*.

The animals were maintained under standard conditions of temperature (25±2 °C) and humidity (60±5%) with an alternating 12 h light/dark cycles. The celecoxib dose was chosen based on therapeutic dose generally used for COX-2 enzyme inhibition in rats. To provide the sufficient inhibition of COX-2 enzyme, all treatments were maintained for 7 days as described in various investigations (11, 12). The experiment was terminated at the end of 7 days and all animals were sacrificed under ether anesthesia. After decapitation, liver, kidney, heart, lung and brain were excised from rats and washed with ice-cold (4°C) saline, labeled and stored at -40°C until homogenization. This study was certified by the Committee of Local Ethics of the Faculty of Medicine of İnönü University (Code:2005-20, Malatya, Turkey).

Determination of Zn^{+2} and Cu^{+2} by Atomic Absorbtion Spectrophotometer

The sample of tissues were first heated in an oven set at 100-105 °C to an accurate weight. An exact amount of tissue (0.2 g) was then digested with 1ml of concentrated nitric acid in a polypropylene tube in an oven adjusted at 65 °C for 2 hrs. Samples were diluted in distilled

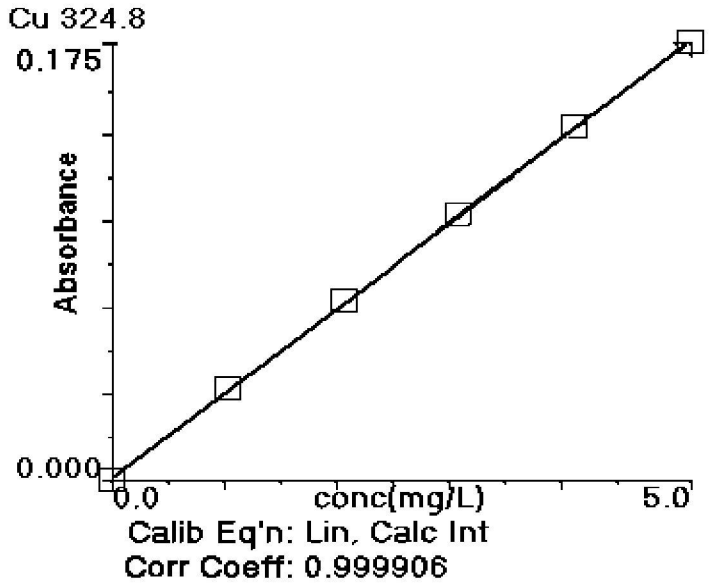
water and measured with Perkin Elmer Analyst 800 atomic absorption spectrometer. The hollow cathod lamps of the respectives elements were operated under standart conditions using their respective resonance lines for Zn^{+2} at 213.9 nm, for Cu^{+2} at 324.8 nm. Samples were volatilized in an air acetylene flame, and the concentration of metal was read directly in micrograms per milliliter (µg/mL) after calibration of the scale with appropriate standards for zinc analysis 0.25-1.5 µg/mL of zinc standard solution, for copper analysis 1-5 µg/mL of copper standard solution. Distilled-deionized water (AAS grade) was used for the preparation of dilutions and standards of the trace element analysis. Results were expressed as micrograms per gram (µg/g) of dried tissue weight (13,14).



ID	Mean Signal (Abs)	Conc. mg/L	Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	-0.035	0.00	51.2
Calib Std 1	0.0484	0.25	0.254	0.00	1.1
Calib Std 2	0.0933	0.5	0.523	0.00	0.0
Calib Std 3	0.1341	0.75	0.767	0.00	1.1
Calib Std 4	0.1773	1.0	1.025	0.00	0.1
Calib Std 5	0.2514	1.5	1.467	0.00	0.2

Correlation Coef.: 0.998726 Slope: 0.16733 Intercept: 0.00587

Figure 1. Calibration curve for Zn^{+2} standarts in concentration ranges of 0.25-1.5 mg/L.



ID	Mean Signal (Abs)	Conc. mg/L	Conc. mg/L	Standard Deviation	%RSD
Calib Blank 1	0.0000	0	-0.034	0.00	18.6
Calib Std 1	0.0366	1.0	1.011	0.00	0.3
Calib Std 2	0.0722	2.0	2.027	0.00	0.3
Calib Std 3	0.1070	3.0	3.023	0.00	0.3
Calib Std 4	0.1412	4.0	4.000	0.00	0.2
Calib Std 5	0.1753	5.0	4.973	0.00	0.0

Correlation Coef.: 0.999906 Slope: 0.03500 Intercept: 0.00120

Figure 2. Calibration curve for Cu^{+2} standarts in concentration ranges of 1-5 mg/L.

Determination of Zn^{+2} in Serum

Serum Zn^{+2} levels were determined by Perkin Elmer Analyst 800 atomic absorption spectrometer with an atomisation in an acetylene-air burner. To determine Zn^{+2} levels, optimum dilutions were prepared with distilled-deionized water (AAS grade). Serum Zn^{+2} levels were expressed in mμg/mL (15).

Determination of Body Weights and Liver Weights

The rats were weighed twice, before begining the treatment and also completion of the experiment. Body weights were recorded as before and after. Liver tissues were dried with blotter and weighed (16). For estimation body and liver weights, Ohaus NV-210 electrobalance was used.

Statistical analysis

Statistical calculations were done by using INSTAT. Results were expressed as means±standard deviation (SD). Differences between groups were examined using one-way analysis of variance (ANOVA) followed by unpaired Student's t-test and Tukey's test.

RESULTS

Tissue Zn^{+2} Levels

The effect of nicotine administration on Zn^{+2} levels are depicted in Table 1. Nicotine treatment increased Zn^{+2} levels significantly in liver, heart and lung ($p<0.05$, $p<0.05$ and $p<0.001$ respectively). However, nicotine plus celecoxib treatment caused significant decreases in Zn^{+2} levels at various degrees ($p<0.01$ and $p<0.001$).

Table 1. Effect of nicotine and nicotine plus celecoxib treatment on Zn^{+2} levels in different tissues (μg/g dry weight).

Group	Liver Mean±SD	Lung Mean±SD	Kidney Mean±SD	Brain Mean±SD	Heart Mean±SD
Control	15.6±0.38	37.3±1.38	23.9±2.34	29.7±2.37	33.4±2.62
Nicotine[a]	20.3±3.60*	42.3±1.09***	26.5±1.65^{ns}	32.8±2.66^{ns}	36.2±3.57*
Nicotine + Celecoxib[b]	15.4±1.27**	31.4±1.77***	23.2±0.97**	25.2±2.25^{ns}	23.8±2.11***

a: Compared to control group, **b:** Compared to nicotine group,
ns: not significant, *p<0.05, **p<0.01, ***p<0.001.

Tissue Cu^{+2} Levels

The effect of nicotine administration on tissue Cu^{+2} levels are depicted in Table 2. Nicotine treatment significantly increased Cu^{+2} levels in lung and heart ($p<0.001$ and $p<0.05$ respectively). It was found that nicotine plus celecoxib treatment decreased Cu^{+2} levels in all tissues when compared to nicotine group.

Table 2. Effect of nicotine and nicotine plus celecoxib treatment on Cu^{+2} levels (μg/g dry weight) in different tissues.

Group	Liver Mean±SD	Lung Mean±SD	Kidney Mean±SD	Brain Mean±SD	Heart Mean±SD
Control	1.4±0.09	4.1±0.04	4.0±0.81	3.6±0.19	3.7±0.13
Nicotine[a]	1.5±0.25^{ns}	4.7±0.23***	4.3±0.18^{ns}	3.7±0.17^{ns}	4.2±0.19*
Nicotin +Celecoxib[b]	1.2±0.05*	3.5±0.23***	3.4±0.22**	3.5±0.16^{ns}	3.4±0.43***

a: Compared to control group, **b:** Compared to nicotine group,
ns: not significant, *p<0.05, **p<0.01, ***p<0.001.

Serum Zn^{+2} Levels

The effect of nicotine administration on rat serum Zn^{+2} levels are depicted in Table 3. Nicotine treatment significantly increased Zn^{+2} level in serum ($p<0.001$). Although there is a relative decrease in Zn^{+2} level depend on nicotine plus celecoxib treatment, it was found not significant statistically.

Table 3. Effect of nicotine and nicotine plus celecoxib treatment on Zn^{+2} levels (μg/ml) in serum.

Group	Serum Mean±SD
Control	1.18±0.08
Nicotine[a]	2.17±0.23***
Nicotin+Celecoxib[b]	2.11±0.11[ns]

a: Compared to control group, **b:** Compared to nicotine group, ns: not significant, ***p<0.001.

Determination of Body Weights

The changes in the body weight of control and treated rats during experimental period were given in Table 4. There was a significant decrease in the body weight of nicotine treated rats when compared to control group. The initial body weights of the rats before tratments were recorded. Then, rats were fed standard pellet diet *ad-libitium*. After 7 days, nicotine group lost approximately 4g body weight. Changes in the levels of body weight was given as mean±SD.

Table 4. Effect of nicotine and nicotine plus celecoxib treatment on body weights (g).

Control	**Before**	**After**
I	276	292
II	311	308
III	191	203
IV	293	303
V	287	293
Mean±SD	271.6±46.8	279.8±43.4
t		2.538
p		p<0.001
Individual difference Mean±SD		8.2±3.2
Nicotine	**Before**	**After**
I	196	200
II	254	250
III	336	330
IV	300	292
V	332	323
VI	325	320
VII	300	300
Mean±SD	291.8±50.6	287.8±47.2
t		2.309
p		p<0.001
Individual difference Mean±SD		-4.0±1.7
Nicotine +Celecoxib	**Before**	**After**
I	282	287
II	251	256
III	277	268
IV	281	277
V	279	285
VI	263	264
Mean±SD	272.1±12.4	272.8±12.1
t		0.2712
p		p<0.01
Individual difference Mean±SD		0.6±2.4

Determination of Liver Weights

Liver weights of rats were decreased in nicotine treated group significantly ($p<0.01$), whereas nicotine plus celecoxib treated rats did not show any significant changes. Effect of nicotine and nicotin plus celecoxib treatment on liver weights was shown in Table 5.

Table 5. Effect of nicotine and nicotine plus celecoxib treatment on liver weights (g/100g body wt).

Group	Liver weights (g/100g body wt) Mean±SD
Control	3.46±0.12
Nicotine[a]	$3.20\pm0.11^{**}$
Nicotine+Celecoxib[b]	3.27 ± 0.12^{ns}

a: Compared to control group, **b:** Compared to nicotine group,
ns: not significant, **$p<0.01$.

DISCUSSION

Trace elements serve as a catalytic component in many enzymes. They are important constituents of metalloproteins and enzymes including lysyl oxidase (required for connective tissue) and cytochrome oxidase etc. (17). The role of trace metallic elements, such as Cu^{+2} in inflammation, is great interest given their function as co-factors in metabolic processes involving connective tissue and immune system and their effect on PG synthesis (17-19). Recent studies show that, nicotine induces alterations in trace element metabolism in rat tissues (7, 20-23). However, it is not clearly known how nicotine is responsible for the observed variation of Cu^{+2} and Zn^{+2} concentrations. Zinc concentrations in liver, lung and heart tissues were found significantly affected by nicotine in our study. It has been demonstrated that Zn^{+2} and Cu^{+2} concentrations are subjected to change in smokers as well. Tobacco smoking can affect antioxidant enzyme activities, thereby indirectly affecting trace element metabolism (10).

Oxidative stress may play a significant physiological role by inducing higher concentrations of the COX-1 and COX-2 enzymes, and hence increasing prostaglandin synthesis. Recently, it has been reported that there was a marked induction of COX-2 isoenzyme during the stress-induced oxidative status in tissues (3, 24). Further, inducible COX-2 enzyme is upregulated in response to acute pro-oxidant exposure (24). We can conclude that the changes in antioxidant enzyme activities may alter the co-factor such as Cu^{+2} and Zn^{+2} concentrations, either directly or indirectly. In the present study, celecoxib ameliorated the alterations in trace element levels. We thought that celecoxib may has beneficial effects against nicotine like pro-oxidants.

Nicotine plays an important inhibitory role on food intake and body weight in humans and animals. Nicotine exposure has reportedly resulted in weight loss which may be a consequence of alterations in metabolic rate (22). Also, nicotine is considered as the major appetite-suppressing component of tobacco and has been suggested to increase metabolic rate and in particular to induce lipid metabolism (23). In our study, the average weight gain by rats was significantly reduced in nicotine-treated rats when compared with normal control rats. The weight loss through the nicotine treatment may be due to stimulation of metabolic pathways and activation of lipoprotein lipase or suppression of lipolysis. Nicotines' anorectic effect depends on various mechanisms. For example, nicotine affects almost all organs of the body by its potent oxidant capacity, which in turn may reflect indirectly on the body weight gain (24). Other studies (24-27), show that acute nicotine treatment increased lipolysis, support the current findings.

Another possible pathway could be the release of leptin by nicotine (16). Release of epinephrine, is also stimulated by nicotine. Epinephrine has been shown to have suppressive effects on feeding, in contrast to norepinephrine that stimulates feeding behavior. It is possible that nicotine-induced leptin release would reduce feeding. Furthermore, it is well known that the activities of gastric vagal and adrenal sympathetic efferents can supressed by cigarette smoking (28).

According to our results, nicotine can alter the trace element levels by inducing the COX-2 pathway. Presumably, nicotine did affect the trace element relationships between tissues as well as components of the free radical defense system. The emerging role of COX-2 in various diseases and the present findings further support the therapeutic potential of celecoxib as a protectant in the treatment of nicotin toxicity.

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