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Protective Effect of Vitamin E and N-Nitro L-Arginine Methyl Ester (L-NAME) on Cigarette Induced Kidney Damage

Sigara ile İndüklenen Böbrek Hasarına Vitamin E ve N-Nitro L-Arginin Metil Esterin (L-NAME) Koruyucu Etkisi

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Abstract

The Cigarette is a material obtained from the leaves of a tobacco plant and believed to be comforting by many people. Cigarette components are known to have adverse effects on renal function because of oxidative stress exposure. Vitamin E is known to be an important antioxidant that protects the organism with various mechanisms and prevents damage at the cellular dimension. L-NAME inhibits nitric oxide (NO) synthase, reducing NO formation and contributing to the reduction of oxidative damage induced by reactive species. In this study, we aimed to investigate the effects of cigarette exposure on rat kidneys and protective effect of vitamin E and L-NAME. For this purpose, Forty five male albino rats were divided into five groups: control, cigarette, cigarette + vitamin E, cigarette + L-NAME, cigarette + vitamin E + L-NAME. The rats were exposed to cigarette smoke by inhalation in special cages and 200 mg/kg BW Vitamin E and/or 50 mg/kg BW L-NAME administered intraperitoneal for 42 days. These procedures were applied every day. At the end of the 42nd day, the kidney tissues and blood of rats were taken for biochemical and histological analysis. Malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) measurements were performed on the kidney tissue homogenate, creatinine and blood urea nitrogen (BUN) were measured in the rat serum. While serum creatinine, BUN and tissue MDA levels significantly increased, GSH levels and CAT activity decreased significantly in cigarette-exposed rats. Serum creatinine, BUN, MDA, GSH and CAT levels were normalized in the treatment groups ($p<0.05$). Histological examinations showed that there was improvement in the treatment groups. It has been observed that kidney damage resulting from cigarette-exposure can be eliminated by the use of vitamin E and L-NAME.

Key words: Cigarette, Kidney damage, L-NAME, vitamin E.

Öz

Sigara, tütün bitkisinin yapraklarından elde edilen ve birçok insan tarafından rahatlatıcı etkisi olduğuna inanılan bir maddedir. Sigara bileşenlerinin, oksidatif stres maruziyeti nedeniyle böbrek fonksiyonu üzerinde olumsuz etkileri olduğu bilinmektedir. E vitamini organizmayı çeşitli mekanizmalarla koruyan ve hücresel boyutta hasarı önleyen önemli bir antioksidan olarak bilinir. L-NAME nitrik oksit (NO) sentaz enzimini inhibe ederek NO oluşumunu azaltır ve reaktif türlerin neden olduğu oksidatif hasarı azaltmaya katkıda bulunur. Bu çalışmada, sigara maruziyetinin sıçan böbrekleri üzerindeki etkisini ve E vitamini ile L-NAME'nin koruyucu etkilerini araştırmayı amaçladık. Bu amaçla, kırk beş erkek albino sıçan beş gruba ayrıldı: kontrol, sigara, sigara + E vitamini, sigara + L-NAME, sigara + E vitamini + L-NAME. Sıçanlar, özel kafeslerde inhalasyon ile sigara dumanına maruz bırakılmış ve 42 gün boyunca her gün tedavi gruplarına 200 mg/kg BW Vitamin E ve/veya 50 mg/kg BW L-NAME intraperitoneal olarak uygulanmıştır. 42. günün sonunda, sıçanların böbrek dokuları ve kanı biyokimyasal ve histolojik analiz için alındı. Sıçan serumunda kreatinin ve kan üre azotu (BUN) ile böbrek dokusu homojenatında malondialdehid (MDA), glutatyon (GSH) ve katalaz (CAT) ölçümleri yapıldı. Sigaraya maruz kalan sıçanlarda serumda kreatinin, BUN ve dokuda ise MDA düzeyleri anlamlı olarak artmışken, GSH düzeyleri ve CAT aktivitesi anlamlı olarak azaldı. Serumda kreatinin, BUN, dokuda ise MDA, GSH düzeyleri ve CAT aktivitesinin tedavi gruplarında normalize olduğu görüldü ($p<0.05$). Histolojik incelemeler, tedavi gruplarında iyileşme olduğunu gösterdi. Bu çalışmada sigara maruziyetinden kaynaklanan böbrek hasarının, E vitamini ve L-NAME kullanımı ile düzeltilebileceği görülmüştür.

Anahtar kelimeler: Sigara, Böbrek hasarı, L-NAME, vitamin E.

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1. Introduction

Smoking is the main cause of preventable morbidity and mortality (Funck-Brentano et al. 2006). More than three million people die worldwide each year due to smoking (Leone 2005). Cigarette smoke contains more than 4000 different chemicals proven to be more than 400 carcinogens; but also various oxidants, such as free radicals and volatile aldehydes, which are probably the main cause of damage to biomolecules (Yeh et al. 2008). The toxic effects of cigarette on the cardiovascular system and the endocrine system are known. It is also shown that oxidant-antioxidant balance affects negatively. Cellular damage occurs in the case of oxidative stress caused by free radicals. Vitamin E has an important role in lipid peroxidation because it is an antioxidant of lipophilic chain breaker. In vitro studies have shown that continuous exposure to cigarette smoke causes vitamin E consumption as well as other antioxidants (Leonard et al. 2003). Nitric oxide (NO) is an oxidant both found in cigarettes and synthesized in the body. Peroxynitrite occurs when the superoxide radical is combined with NO. The resulting peroxynitrite also oxidizes the lipids and proteins, causing cellular damage The superoxide radical forms peroxynitrite with NO. The resulting peroxynitrite also oxidizes the lipids and proteins, causing cellular damage (Modlinger et al. 2004). Depending on the amount of NO increased, the free radical ratio in the body increases. L-NAME inhibits the NO synthase enzyme complex that catalyzes NO synthesis (Moncada et al. 1991).

This study investigated the protective effect of vitamin E and L-NAME on the deterioration of oxidant-antioxidant balance resulting from the increase of free radicals due to the cigarette.

2. Materials and Methods

2.1 Experimental Issues

The ethical committee report for the study was taken from the Eskişehir Osmangazi University (ESOGU) Local Ethics Committee (HADYEK). Within the scope of the study, 45 male albino rats were divided into five groups; control, cigarette, cigarette + vitamin E, cigarette + L-NAME, cigarette + vitamin E + L-NAME. Exposure to cigarettes was carried out by specially designed inhalation cage. The inhalation cage comes from two separate parts. The smoke produced by the combustion of the first cigarette smoker in the special measurements was transferred by means of a motor with the aid of a pipe to the other cage where the rats were located. 200 mg/kg BW Vitamin E and 50 mg/kg BW L-NAME were administered intraperitoneally for 42 days in the treatment groups. On day 43, tissue and blood samples were taken from the rats. MDA, GSH levels and CAT activity were measured in tissue homogenate. In addition, serum creatinine and BUN levels were measured. Protein levels in tissue homogenate were measured by biuret method (Gornall et al. 1949).

2.2 Measurement of Lipid Peroxidation

Lipid peroxidation in body fluids or cells is considered to be indicative of oxidative stress. MDA measurement is also performed to determine lipid peroxidation. Tissue MDA measurement was performed by Ohkawa et al. (1979).

2.3 Measurement of Antioxidant / Antioxidant Enzyme

GSH was measured in tissue homogenate according to the method reported by Srivastava and Beutler (1968). The CAT enzyme activity, which catalyzes the breakdown of water and molecular oxygen of hydrogen peroxide, was carried out according to the method reported by Aebi (1984).

2.4 Measurement of Kidney Function Parameters

Serum creatinine and BUN measurements were measured with Roche Cobas-c 501 autoanalyzer located in ESOGU biochemistry laboratory.

2.5 Statistical Evaluation

Statistical analysis was performed by one way variance analysis (ANOVA) and group comparisons were performed by Tukey HSD test. Analysis results are shown as mean ± S.E.M.

3. Results

Oxidative stress was induced in the kidney with cigarette smoking and the renal damage was tried to be eliminated with vitamin E and L-NAME. A significant increase in the level of MDA was observed in the cigarette group compared to the control group ($p<0.001$). In the groups treated with vitamin E and L-NAME, MDA levels decreased significantly compared to the cigarette group ($p<0.001$). When cigarette group compared to the control group, it was observed that the level of GSH, an important antioxidant, decreased ($p<0.01$). Cigarette + vitamin E and cigarette + vitamin E + L-NAME groups showed a significant increase in GSH level when compared to the cigarette group(respectively, $p<0.001$, $p<0.01$). As a result of statistical evaluation of CAT enzyme activity, CAT activity decreased significantly when compared to the control group and the cigarette group ($p<0.01$). CAT activity was significantly increased in the treatment groups compared to the cigarette group ($p<0.05$). The results are shown in Tab. 1.

Serum creatinine and BUN levels were measured in the blood of rats and presented in Tab. 2. Serum creatinine levels were increased in the cigarette group compared to the control group ($p<0.001$). Serum creatinine levels were significantly reduced in the groups treated with vitamin E and L-NAME compared to the cigarette group ($p<0.001$). BUN levels in cigarette group were significantly higher than the control group ($p<0.001$). However, a significant decrease in BUN values were observed with the application of vitamin E and L-NAME ($p<0.001$).

Table 1. MDA, GSH levels and CAT activity in the rat kidney tissue.

Group (N=9)	Kidney Tissue Homogenesis		
	MDA (nmol/mg protein)	GSH (µmol/mg protein)	CAT (U/mg protein)
Control	1.238 ± 0.051	0.511 ± 0.023	58.979 ± 2.924
Cigarette	3.388 ± 0.216*	0.399 ± 0.012$^{\#}$	53,279 ± 2.992^{+}
Cigarette+Vitamin E	0.933 ± 0.045**	0.666 ± 0.045$^{\#\#}$	61.603 ± 5.932^{++}
Cigarette+L-NAME	1.355 ± 0.128**	0.438 ± 0.035	58.818 ± 2.213^{++}
Cigarette+Vitamin E +L-NAME	1.271 ± 0.125**	0.586 ± 0.033$^{\#\#\#}$	61.839 ± 2.677^{++}

*: p<0.001; compared with the control group, **: p<0.001; compared with the cigarette group, $^{\#}$: p<0.01; compared with the control group, $^{\#\#}$: p<0.001; compared with the cigarette group, $^{\#\#\#}$: p<0.01; compared with the cigarette group, $^{+}$: p<0.01; compared with the control group, $^{++}$: p<0.05; compared with the cigarette group.

Table 2. Serum creatinin and BUN levels.

Group (N=9)	Serum	
	Serum Creatinin (mg/mL)	BUN (mg/mL)
Control	0.3025 ± 0.005	19.688 ± 0.371
Cigarette	0.435 ± 0.008*	25.463 ± 0.760$^{\#}$
Cigarette+Vitamin E	0.330 ± 0.007**	19.250 ± 0.354$^{\#\#}$
Cigarette+L-NAME	0.366 ± 0.002**	18.833 ± 0.816$^{\#\#}$
Cigarette+Vitamin E+L-NAME	0.350 ± 0.002**	19.129 ± 0.321$^{\#\#}$

*: p<0.001; compared with the control group, **: p<0.001; compared with the cigarette group, $^{\#}$: p<0.001; compared with the control group, $^{\#\#}$: p<0.001; compared with the cigarette group.

3.1 Histological Examination

Histological examination of kidney tissue revealed damage to the cigarette group. Damage to the cortex and medullary was determined. A decrease in cellular damage was observed in the cortex, medulla, and kidney bodies due to the administration of Vitamin E and L-NAME. In the treatment group given Vitamin E, the damage caused by cigarette smoking was seen to have improved.

Examination of kidney tissues by light microscopy is shown in Fig. 1. In control group (**A**); Cortex and medulla were observed normally. Kidney bodies, proximal tubules (pt) and distal tubules (dt) were seen in normal histological structure. In cigarette group (**B**); Intense damage was observed in cortex and medulla. The decrease in Bowman's range and glomerular damage was clear in the kidney body. In addition, tubular dilatation and damaged tubule structures were observed. In cigarette+vitamin E group (**C**); Reduced damage was observed in cortex and medulla. It was seen in the histologic structure close to normal in kidney bodies, macula densa structure, proximal tubule (pt) and distal tubule (dt) structures. In cigarette+L-NAME group (**D**); Decreased damage in the cortex and in the medulla was remarkable. Kidney bodies were observed close to normal. Apart from a few tubular injuries and cellular infiltration in the interstitial area, histologic structures near the normal were seen with proximal tubules (pt) and distal tubule (dt) structures. In cigarette+Vitamin E+L-NAME group (**E**); the appearance of reduced damage to the cortex and medullary was remarkable. Kidney bodies were normal. Apart from a few tubular injuries and cellular infiltration in the interstitial area, the histologic structure was normal with proximal tubules and distal tubule structures.

4. Discussion

Oxidative stress in the body is constantly affected by many external factors that are sources of free radicals. In kidney injury, deterioration of oxidative stress mechanisms has an important place (Sureshbabu et al. 2015). Tobacco and tobacco products are among the external factors that increase the number of free radicals. It is also a general idea that these products are also effective in acute and chronic renal damage. Studies conducted by Sánchez et al. (2016) have shown that kidney damage occurs due to tobacco use. This result is also valuable because it correlates with cotinine that a metabolite of nicotine. It is known that cigarette, the most important tobacco products, contains nicotine as well as free radical species.

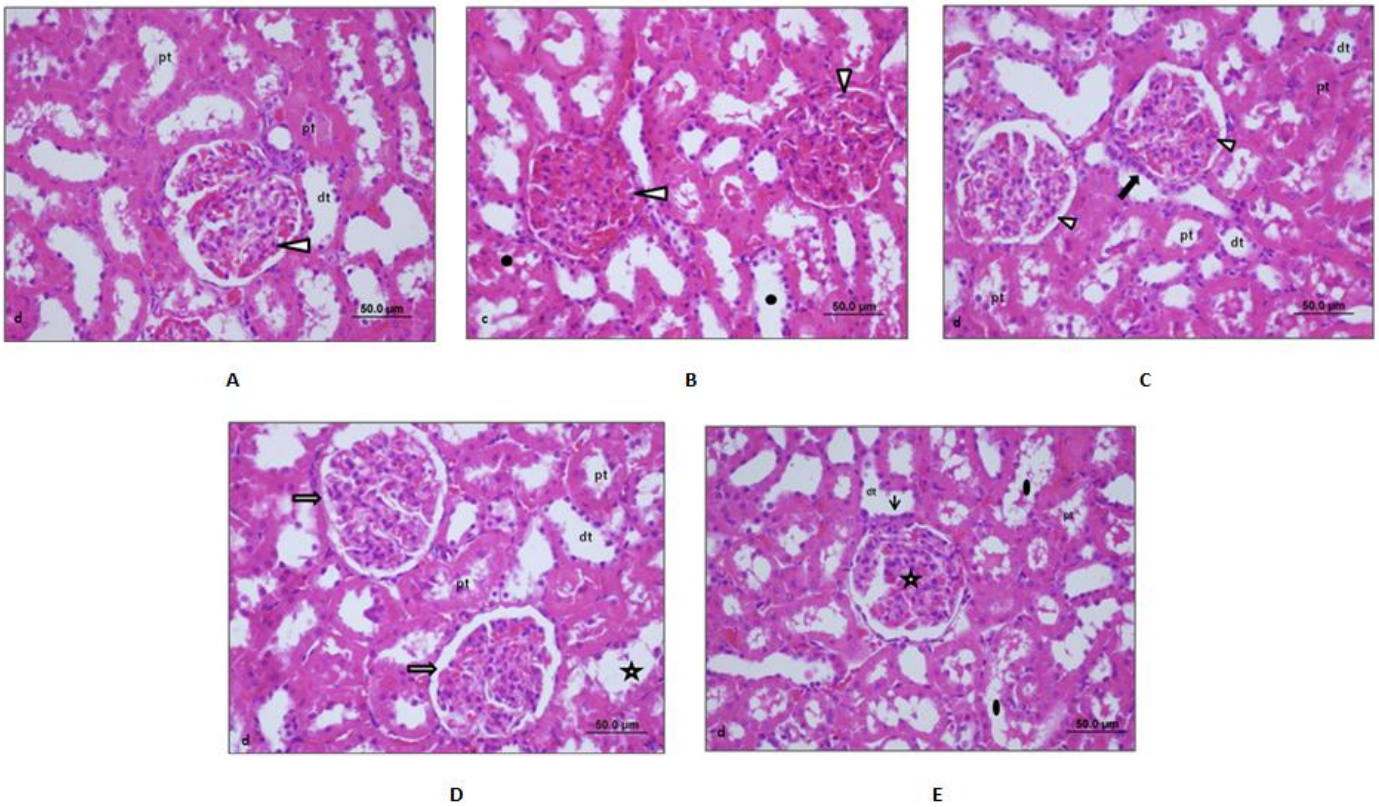

Figure 1: Light microscopic examination of rat kidney tissues (bar: 50.0µm).

The study by Sanchez et al. (2016) reported that there was a significant association between cotinine and MDA, a lipid peroxidation indicator. It has been reported that kidney damage is caused by smoke exposure, which is associated with oxidative stress (Hussain et al. 2016). This study also found significant increase in MDA level due to the cigarette. In order to decrease the MDA level, vitamin E and L-NAME, which are important antioxidants, have been administered and positive results have been obtained. Studies have shown that there is an inverse relationship between GSH and oxidative stress. This suggests that GSH is an important cellular defence mechanism against oxidative damage. GSH is an important antioxidant that is effective in clearing free radicals in the kidney (Vaziri et al. 2000; Ganafa et al. 2002; Modlinger et al. 2004). Decreased GSH levels due to cigarette exposure have been increased to normal levels with vitamin E from natural antioxidants. However, statistically significant results were not obtained for the L-NAME application. CAT from enzymatic antioxidants plays an important role in the elimination of oxidative stress that occurs in tissues. CAT activity is one of the markers used to determine oxidative stress in renal disease patients (Modaresi et al. 2015). Tucker et al. (2013) reported that the results of CAT as an antioxidant marker in the kidney were inconsistent. As a result of cigarette exposure in our study, a decrease in CAT activity was observed. With vitamin E and L-NAME administration, CAT activity significantly increased in the treatment groups compared to the cigarette group. It has been demonstrated that oxidative stress in the kidney is related with serum creatinine and BUN levels.

Serum creatinine and BUN levels increase indirectly proportional to oxidative stress (Saleh and El-Demerdash 2005; Hussain et al. 2016). Serum creatinine and BUN levels were increased due to kidney damage caused by the cigarette exposure. This increase may be related to impaired renal function. The use of vitamin E in support of antioxidant defence in eliminating oxidative damage in the kidney reduced serum creatinine and BUN levels. NO adversely affects kidney function in the case of oxidative stress. L-NAME inhibits NO synthesis. In this case, reduction of serum creatinine and BUN levels in treatment group may be explained by the inhibition of NO synthesis by L-NAME.

In daily life, there are many factors that trigger oxidative stress in our environment. These include polluted air, exhaust fumes and cigarettes. Increased oxidative stress due to cigarette can cause nephrotoxicity. It is important to measure renal function parameters as well as examined of lipid peroxidation and antioxidant defence in order to determine oxidative stress in the kidney. Vitamin E, an important functional property especially in lipoproteins, is an efficient antioxidant in reducing oxidative damage. L-NAME, which plays a role in the inhibition of NO synthase enzyme, may stop the progress of oxidative stress by decreasing NO amount. As a result, we think that oxidative stress caused by the cigarette in kidneys can be eliminated by the application of vitamin E and L-NAME.

Conflicts of Interest: No conflict of interest was declared by the authors.

References

Aebi H. 1984. Catalase in vitro. In Methods in Enzymology, 105: 121–126.

Funck-Brentano C, Raphaël M, Lafontaine M, Arnould JP, Verstuyft C, Lebot M, Roussel R. 2006. Effects of type of smoking (pipe, cigars or cigarettes) on biological indices of tobacco exposure and toxicity. Lung Cancer, 54/1: 11–18.

Ganafa AA, Socci RR, Eatman D, Silvestrova N, Abukhalaf IK, Bayorh MA. 2002. Acute inhibition of glutathione biosynthesis alters endothelial function and blood pressure in rats. European Journal of Pharmacology, 454/2–3: 217–223.

Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry, 177/2: 751–766.

Hussain T, Al-Attas OS, Alrokayan SA, Ahmed M, Al-Daghri NM, Al-Ameri S, Sumague TS 2016. Deleterious effects of incense smoke exposure on kidney function and architecture in male albino rats. Inhalation Toxicology, 28/8: 364–373.

Leonard SW, Bruno RS, Paterson E, Schock BC, Atkinson J, Bray TM, Cross CE, Traber MG 2003. 5-Nitro-γ-tocopherol increases in human plasma exposed to cigarette smoke in vitro and in vivo. Free Radic Biol Med, 35/12: 1560–1567.

Leone A. 2005. Biochemical markers of cardiovascular damage from tobacco smoke. Current Pharmaceutical Design, 11/17: 2199–2208.

Modaresi A, Nafar M, Sahraei Z. 2015. Oxidative stress in chronic kidney disease. Iranian Journal of Kidney Diseases, 9/3: 165–179.

Modlinger PS, Wilcox CS, Aslam S. 2004. Nitric oxide, oxidative stress, and progression of chronic renal failure. In Seminars in Nephrology, 24/4: 354–365.

Moncada SRMJ, Palmer RML, Higgs EA 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews, 43/2: 109–142.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95/2: 351–358.

Saleh S, El-Demerdash E. 2005. Protective Effects of L-Arginine against Cisplatin-Induced Renal Oxidative Stress and Toxicity: Role of Nitric Oxide. Basic & Clinical Pharmacology & Toxicology, 97/2: 91–97.

Sánchez MH, Vicente LV, Parras FS, Casanova AG, Pescador M, Prieto M, Morales AI. 2016. Oxidative stress role in the development of kidney injury produced by tobacco. Toxicology Letters, 258:255.

Srivastava SK, Beutler E. 1968. Accurate measurement of oxidized glutathione content of human, rabbit, and rat red blood cells and tissues. Analytical Biochemistry, 25: 70–76.

Sureshbabu A, Ryter SW, Choi ME. 2015. Oxidative stress and autophagy: crucial modulators of kidney injury. Redox Biology, 4: 208–214.

Tucker PS, Dalbo VJ, Han T, Kingsley MI. 2013. Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers, 18/2: 103–115.

Vaziri ND, Wang XQ, Oveisi F, Rad B. 2000. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Hypertension, 36/1: 142–146.

Yeh CC, Barr RG, Powell CA, Mesia-Vela S, Wang Y, Hamade NK, Santella RM. 2008. No effect of cigarette smoking dose on oxidized plasma proteins. Environmental Research, 106/2: 219–225.