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SODIUM SELENITE ATTENUATES NICOTINE-INDUCED OXIDATIVE STRESS IN RAT

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ABSTRACT

Nicotine, a major toxic component of cigarette smoke has been identified as a major risk factor for different diseases. In the present study, we evaluated the protective effects of sodium selenite on lipid peroxidation and antioxidants status against nicotine treated liver and kidney toxicity. A group of male Wistar rats were induced by subcutaneous injection of vehicle, nicotine (2mg/kg body weight/day) and nicotine plus sodium selenite (0.5 mg/kg of diet) for the period of 15 days. Measurement of biochemical marker enzyme like lactate dehydrogenase (LDH), lipid peroxidation (MDA), conjugated dienes (CD) and antioxidants were used to monitor the antiperoxidative effect of sodium selenite. The decreased biochemical marker enzyme as well as increased lipid peroxides and conjugated dienes in liver and kidney of nicotine treated rats was accompanied by a significant decrease in the levels of glutathione (GSH and GSSG) and the activities of glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). Dietary co-administration of sodium selenite significantly supplements the biochemical marker enzyme, lipid peroxidation and enhanced the antioxidant status. The results of the present study suggest that sodium selenite exert its protective effect against nicotine-induced liver and kidney toxicity by modulating the biochemical marker enzyme, lipid peroxidation and augmenting antioxidant defense system.

KEYWORDS: Nicotine, Sodium selenite, Tissues, Oxidative stress.

INTRODUCTION

Nicotine, a pharmacologically active substance in tobacco, is generally regarded to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer (Jung et al. 2001). Nicotine has been reported to induce oxidative stress both in vivo and in vitro (Suleyman et al. 2002). Oxidative stress arises when there is an imbalance between oxygen free radical (OFR) formation and scavenging by antioxidants. Increased OFR production has been directly linked to oxidation of cellular macromolecules, which may cause direct lung injury or induce a variety of cellular responses through the generation of secondary metabolic reactive species (Chiarugi 2003). Nicotine-induced alterations in hepato – renal phosphate activities have also been reported (Das Gupta and Ghosh 1993). The mechanisms of free radical generation by nicotine are not clear. However, it has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anions and hydrogen peroxide (Yildiz et al. 1999).

Multiple studies have shown a protective effect of vitamin C on lung function. Increased vitamin C intake is associated with decreased chronic obstructive pulmonary disease in adult smokers (Sargeant et al. 2000). In our laboratory, studies reported that vitamin E and reduced glutathione (GSH) are able to ameliorate nicotine-induced oxidative stress in tissues (Neogy et al. 2008; Dey and Roy 2010).

Selenium is a structural component of several enzymes including glutathione peroxidase (GPx) and thioredoxine (Perottoni et al. 2004), which play a key role in the cellular oxidative defense and have been shown to be induced by oxidative stress (Lechner et al. 2002). In this year, there has been a great deal of studies carried out on selenium metabolism (Shi et al. 2004). In most of these studies selenium was administered to experimental animals in sodium selenite form (Ates et al. 2008). Many efforts have been undertaken in evaluating the relative antioxidant potential of selenium against environmental pollutants which cause health problems (El-Demerdash 2001; El-Demerdash 2004; Ersteniuk 2004). Selenium has been shown to prevent damage induced by free radicals to the unsaturated fatty acid of subcellular membranes (Thomas et al. 1990). It has been also observed that selenium administration reduces alcohol-and nicotine-induced toxicity in the testis (Seema et al. 2007).

In this present investigation, therefore, we have evaluated the effects on nicotine-induced oxidative damage in liver and kidney of the dietary co-administration of sodium selenite.

MATERIALS AND METHODS

Chemicals

Nicotine tartrate, sodium selenite and other fine chemicals were purchased from Sigma Chemical Company, USA. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt Ltd (SRL), India, and were of analytical grade.

Animals and diet

Adult male albino rats (n=36) of Wistar strain of body weight 100-120 *g* were obtained. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Vidyasagar University, Midnapore, and were housed in polypropylene cages and fed standard pellet diet (Hindusthan Lever Ltd, India) for 1 week and water ad libitum. Animals were divided into three groups and each group consisting 12 animals.

Mode of treatment

Animals were divided into three groups of almost equal average body weight of twelve animals each. The animals of two groups were induced by subcutaneous injection with nicotine tartrate (dissolved in 0.9% physiological saline) at a dose of 2mg / kg body weight per day for 15 days, as described earlier (Dey and Roy 2010). The animals of one of the nicotine treated groups serving as the supplemented group supplied sodium selenite (0.5 mg/kg of diet) for a period of 15 days. The animals of the remaining group received only the vehicle (0.9% physiological saline), served as control.

Animals sacrifice and sample preparation

After completion of drug treatment the animals were fasted overnight prior to sacrifice by the use of anesthesia. The intact liver and kidney were dissected out and adhering blood and tissue fluid were blotted dry weighted and kept at -20^{0}C prior to homogenization and analysis.

Analytical methods

Lipid peroxidation was measured according to the method of Ohkawa et al. (1979). Malondialdehyde (MDA) was determined from the absorbance of the pink coloured product (TBARS) of thiobarbituric acid-MDA reaction, at 530 nm. The reaction of MDA with TBA

has been widely adopted as a sensitive method of lipid peroxidation in animal tissues. Conjugated dienes was measured according to the method of Slater (1980). Lipids were extracted with chloroform/methanol and the lipid residue was dissolved in cyclohexane and absorbance of the formed hydroperoxide is noted at 233 nm in spectrophotometer. The activity of lactate dehydrogenase (LDH) was measured by the method of Young et al. (1975).

GSH was measured according to the method of Griffith (1980). GSSG was also assayed after derivatization of GSH with 2 vinylpyridine. GSSG was measured by the method of Griffith (1980).

Catalase activity was determined at room temperature by using a slightly modified version of Aebi (1984). The molar extinction coefficient of 43.6Mcm^{-1} was used to determine CAT activity. One unit of activity is equal to the millimoles of H_2O_2 degraded per minute per milligram of protein. SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto- oxidation by SOD according to the method Marklund & Marklund (1974).

The rate of oxidation of reduced glutathione (GSH) by H_2O_2 as catalyzed by the glutathione peroxides (GPx) present in the homogenate is assayed for the measurement of enzyme activity. Glutathione peroxidase activity was measured according to method of Paglia and Valentine (1967). The activity of glutathione reductase was measured by the method of Miwa (1972). Glutathione S-transferase activity was measured according to the method of (Habig et al. 1974)

Total protein of plasma and tissues was estimated according to the method of Lowry et al. (1951).

Statistical Analysis

The data were expressed as mean ± S.E.M. Comparisons of the means of control, nicotine and nicotine with sodium selenite group were made by two-way ANOVA with multiple comparison 't'-test, $P<0.05$ as a limit of significance.

RESULTS

MDA levels were significantly increased in liver and kidney by 76.47% and 37.28%, respectively, as compared to the control group. Supplementation with sodium selenite showed significant diminution of MDA content in liver by 38.66% and in kidney by 16.04%,

respectively, as compared to nicotine treated group (Figure-1). On the other hand, the levels of conjugative dienes were significantly increased in liver and kidney by 156.35 % and 196.73%, respectively, when compared with control group (Figure-2). Significantly decreased levels of conjugated dienes were seen after supplementation with sodium selenite in liver by 34.38% and in kidney by 25.16%, respectively, when it compared with nicotine treated groups of animals (Figure-2).

The activity of biochemical marker enzyme LDH was significantly lower in nicotine treated rats compared with control in liver and kidney by 39.60% and 29.47%, respectively (Figure-3). After supplementation with sodium selenite, the LDH activity elevated in liver by 48.40% and in kidney by 25.37%, respectively, in comparison to nicotine treated animals (Figure-3).

The activities of SOD and CAT were significantly reduced in liver by 59.21% and 54.54%, and in kidney by 62.43% and 51.51%, respectively, in response to nicotine treatment when compared with control group (Figure 4 & 5). Sodium selenite supplementation increased the activity of SOD in liver by 74.19% and in kidney by 70.29%, respectively, in comparison to nicotine administered animals (Figure-4). But the supplementation with sodium selenite, the activity of CAT elevated in liver by 70.0% and in kidney by 62.5%, respectively, when compared with nicotine treated animals (Figure-5).

The levels of GSH and GSSG were significantly diminished in liver by 51.56% and 55.2% and in kidney by 35.04% and 54.05%, respectively, in nicotine administered animals (Figure-6 & 7). Supplementation with sodium selenite showed significant elevation of GSH content in liver by 43.14% and in kidney by 27.38%, respectively, as compared to nicotine treated group (Figure-6). The level of GSSG was seen after supplementation with sodium selenite in liver by 69.64% and in kidney by 17.64%, respectively, in comparison to nicotine administered animals (Figure-7).

The activities of GPx, GR and G-S-T were significantly decreased in liver by 63.05%, 53.63% and 34.13% and in kidney by 50.42%, 45.44% and 32.25%, respectively, in response to nicotine treatment when compared with control group (Figure 8, 9 & 10). Sodium selenite supplementation increased the activity of GPx in liver by 109.6% and in kidney by 40.57%, respectively, in comparison to nicotine administered animals (Figure-8). But the supplementation with sodium selenite the activity of GR elevated in liver by 63.87% and in kidney by 36.75%, respectively, when compared with nicotine treated animals (Figure-9). On

the other hand, significantly increased activity of G-S-T was seen after supplementation with sodium selenite in liver by 31.20% and in kidney by 23.38%, respectively, in comparison to nicotine administered animals (Figure-10).

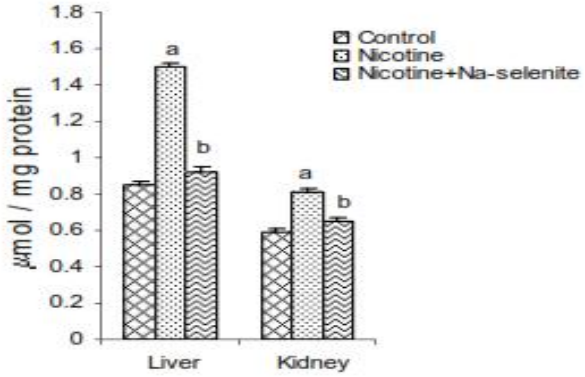

Figure 1: Changes the MDA content in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

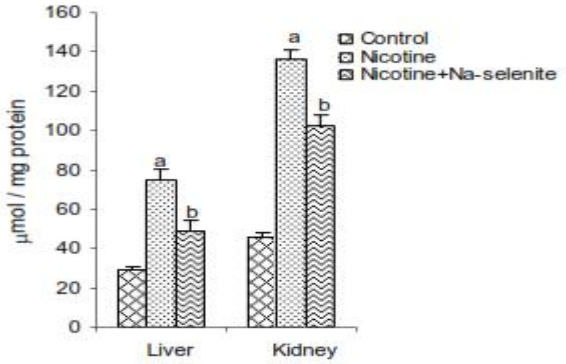

Figure 2: Changes the CD content in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

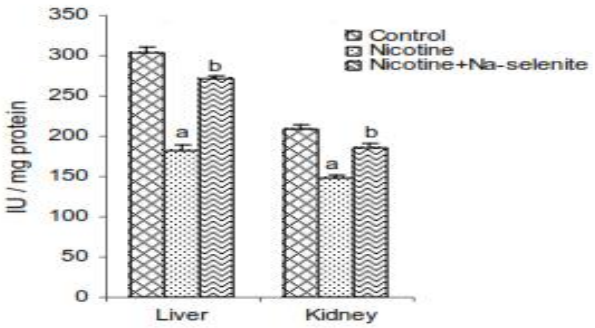

Figure 3: Changes the LDH activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

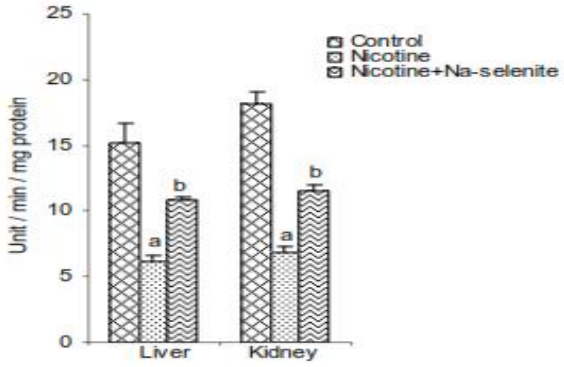

Figure 4: Changes the SOD activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine

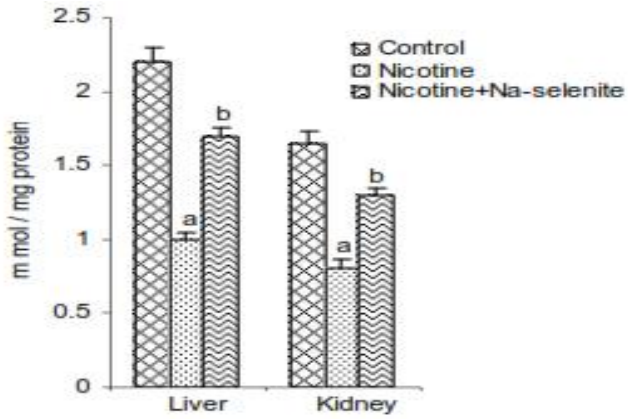

Figure 5: Changes the CAT activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

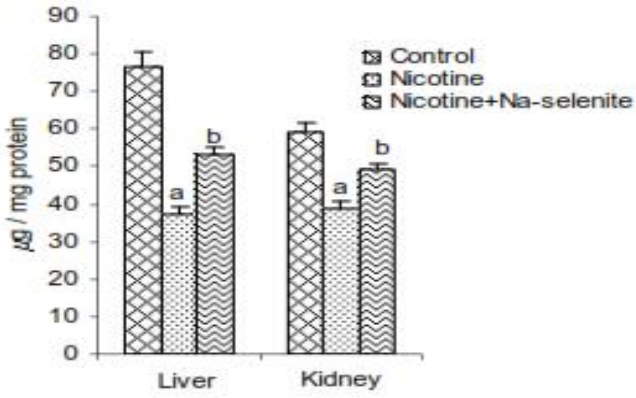

Figure 6: Changes the GSH content in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

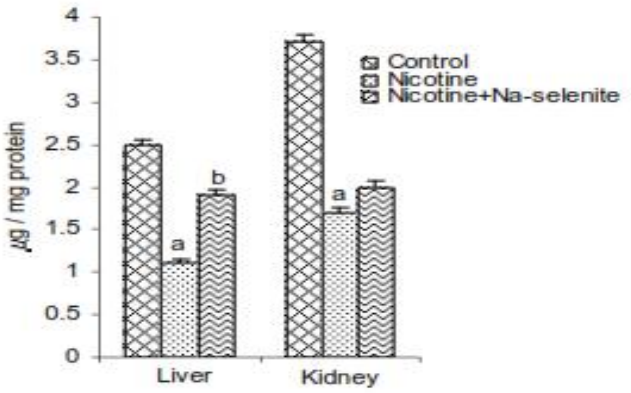

Figure 7: Changes the GSSG content in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

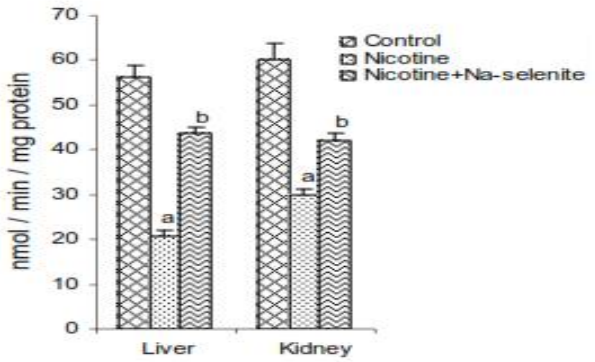

Figure 8: Changes the GPx activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

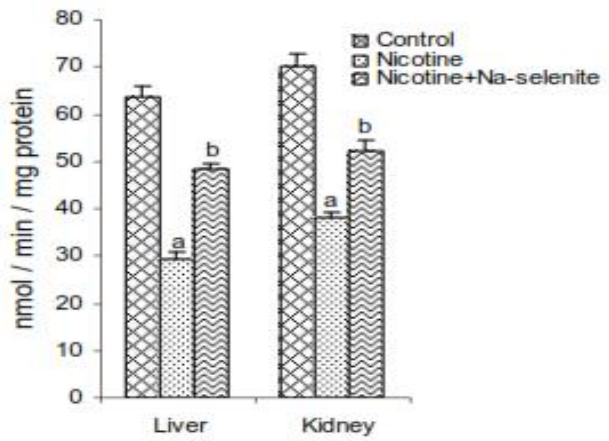

Figure 9: Changes the GR activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

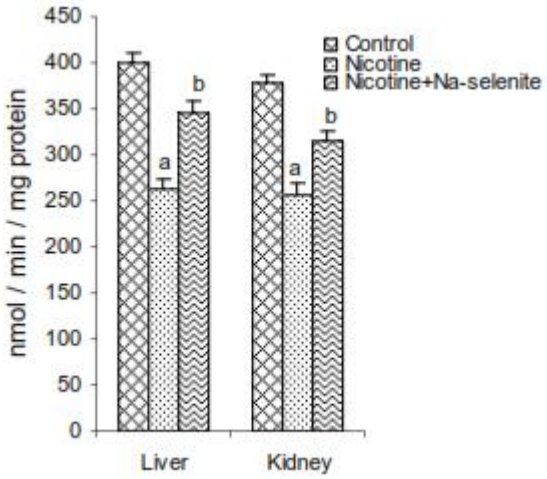

Figure 10: Changes the GST activity in liver and kidney after co-administration of sodium selenite in nicotine treated rats. Data represents mean ± SE, N=12. [a] p<0.05 compared to control, [b] p<0.05 compared to nicotine.

DISCUSSION

Enhanced lipid peroxidation and conjugated dienes in liver and kidney is a characteristic observation in nicotine treated rats. Nicotine, a potent carcinogen, plays a key role in the toxicity of liver, kidney and lungs (Husain et al. 2001). The mechanism of free radical generation by nicotine is not clear. However, it has been reported that nicotine is chemotactic for polymorphonuclear (PMN) leucocytes and enhances the responsiveness of PMN leucocytes to activated complement C5a, thus generating oxygen free radical (Wetscher et al. 1995). Further, nicotine disrupts the mitochondrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide (Yildiz et al. 1999). Chronic nicotine administration also induces cytochrome P_{450} type (CYP1A1 in lung) as well as generates free radicals and exerts oxidative tissue injury (Iba and Fung 1999). Thus, the decreased activity of marker enzyme LDH and increased the level of MDA and conjugate dienes in liver and kidney of nicotine treated rats in the present study may be due to excessive generation of free radicals by nicotine. However, supplementation of sodium selenite reduced the levels of lipid peroxidation products and increased the activity of marker enzyme.

Numerous studies have shown the importance of SOD in protecting cells against oxidative stress (Huang et al. 1997). Previous reports have shown the decreased activity of SOD and CAT in the tissues of nicotine treated rats (Ashakumary and Vijayammal 1991). Thus decrease in the activity of SOD observed in the present study could be due to a feed back inhibition or oxidative inactivation of enzyme protein due to excess ROS generation.

Catalase, which acts as preventative antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation (Dey and Roy 2010). The inhibition of CAT activity is suggestive of enhanced synthesis of superoxide anion during the ingestion of nicotine since superoxide anion is a powerful inhibitor of catalase. Supplementation of selenium enhanced the activity of scavenging antioxidant enzymes (Agarwal and Behari 2007). Studies have shown that selenium pretreatment protected the brain against restraint stress-induced oxidative damage (Atif et al. 2008).

GSH plays a crucial role in protecting the liver and kidney from oxidative stress by detoxifying exogenous toxicants and quenching reactive oxygen species (ROS). High concentration of GSH is found in cells as the major antioxidants defense, especially in regulating the extent and duration of oxidative 'burst' (Abidi et al. 1999). Previous studies have suggested that superoxide anion and hydrogen peroxide are the main source of nicotine-induced free radicals depleting the cellular antioxidant (Helen et al. 2000). Consistent with these reports, we also observed decreased the levels of GSH and GSSG in the liver and kidney of nicotine-treated rats. Studies have shown that glutathione content increased significantly in selenium-treated hypoxic cells (Sarada et al. 2008). We have also observed that sodium selenite have an important role to increase the level of glutathione in response to nicotine.

GPx has a well-established role in protecting cells against oxidative injury. GPx utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and hydrogen peroxide (Ray and Husain 2002). Therefore the excess H_2O_2 and lipid peroxides generated during nicotine ingestion are efficiently scavenged by GPx activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanisms during nicotine ingestion. There are alternative functions for GSH in cellular metabolism independent of its antioxidant properties. GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione-s-transferase (Dey and Roy 2010). Previous reports showed that cigarette smoking results in decreased activities of scavenging enzymes and an inhibition of the enzyme GR (Ozokutan et al. 2005; Erat et al. 2007). Husain et al. (2001) have reported that chronic administration of ethanol and nicotine decreased the level of GSH and activities of GPx, SOD and CAT in the lung and kidney. In the present study, the depletion of GSH, GPx, GR, GST, SOD and CAT in liver and kidney of nicotine treated rats may be due to enhanced utilization during detoxification of nicotine.

Coadministration of sodium selenite significantly enhanced the antioxidant status in liver and kidney of nicotine treated rats and attenuates the damaging effects. It is reported that vitamin C can completely prevent lipid peroxidation in human plasma exposed to cigarette smoke (Frei et al. 1991). Recently, it indicates that comparatively large doses of vitamin C may protect the smokers from cigarette smoke–induced oxidative damage and associated degenerative diseases (Panda et al. 2000). Helen and Vijayammal (1999) reported that an intake of a mega dose of vitamin C can protect the liver from oxidant damage caused by cigarette. In our laboratory, recent studies reported that vitamin E and GSH are able to ameliorate nicotine-induced oxidative stress in tissues (Dey and Roy 2010; Neogy et al. 2008).

These findings indicate that nicotine treatment at the present dose and duration induces oxidative damage of both liver and kidney. These changes can be attenuated by the dietary coadministration of sodium selenite. However, more detailed studies are needed to know the exact mechanism of nicotine-induced oxidative damage. The study with varied dose and duration and other free radical scavengers and antioxidative supporters are also valuable to get the means by which toxic impacts of nicotine can be attenuated.

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