

## Selenium and Vitamin E Modulates Cigarette Smoke Exposure-Induced Oxidative Stress in Blood of Rats

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**Abstract** Cigarette smoke contains about 5,000 chemicals that include organic and metallic compounds. The current study was undertaken to investigate the effects of selenium and vitamin E on oxidative stress-induced damage in rats exposed to cigarette smoke. Forty male rats were equally divided into four groups. The first and second groups were used as control and cigarette smoke groups, respectively. Selenium was administered to rats constituting the third group for 27 days. The Se and vitamin E combination was given to animals in fourth group for 27 days. All groups except the control, were exposed to cigarette smoke starting at the third day of the experiment and continuing for 27 days. The blood samples from all groups were taken at the end of 27 days. Plasma lipid peroxidation, triacylglycerol, and total cholesterol levels were higher in the cigarette smoke group than in the control, although erythrocytic superoxide dismutase and glutathione peroxidase activities were lower in the cigarette smoke group than in the control. The plasma lipid peroxidation, triacylglycerol, and total cholesterol levels were lower in cigarette smoke+Se+VE group than in the cigarette smoke group, although erythrocytic superoxide dismutase activity and glutathione peroxidase activity in selenium and vitamin E-administered groups were higher than in the exposed to cigarette smoke group. High-density lipoprotein-cholesterol level was not affect by selenium and vitamin E administrations. In conclusion, selenium and vitamin E seem to have protective effects on the cigarette smoke-induced blood toxicity by supporting the enzymatic antioxidant redox systems.

**Keywords** Selenium · Antioxidant · Oxidative stress · Cigarette smoke · Erythrocytes

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## Abbreviations

GSH-Px	Glutathione peroxidase
HDL-C	High-density lipoprotein-cholesterol
LP	Lipid peroxidation
MDA	Malondialdehyde
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
Se	Selenium
SOD	Superoxide dismutase
TAG	Triacylglycerol
TC	Total cholesterol
VE	Vitamin E

## Introduction

Cigarette smoke contains numerous organic and metallic compounds emitted as gases and condensed tar particles, many of them being oxidants and prooxidants, capable of producing reactive oxygen species (ROS), thus enhancing lipid peroxidation (LP) in cell membranes [1]. These chemically reactive oxygen species are known to be present or formed in cigarette smoke which may lead to modification of biological macromolecules [2]. The relatively high levels of ROS in cigarette smoke can deplete antioxidants and initiate peroxidation of the polyunsaturated fatty acids (PUFAs) and modification of proteins and nucleic acids in biological systems [3]. Aside from smoke-borne organic radicals, cigarette smoking can also induce endogenous production of ROS including superoxide radical, hydrogen peroxide, and hydroxyl radical in various cells [4, 5]. Exposure to cigarette smoke is also associated with increased oxidative DNA damage [6] and increased change of lipid profiles [7].

Erythrocytes are extremely susceptible to oxidative damage induced by ROS because erythrocytes contain hemoglobin and PUFAs which can be readily be peroxidized [8, 9]. Lipid peroxidation causes injury to cell and intracellular membranes and may lead to cell destruction and subsequently cell death [4, 5]. Erythrocytes are protected by antioxidants from peroxidative damage [9]. These antioxidants build up an efficient defense system, and an important part of this system consists of two major enzymes: superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). SOD converts superoxide radicals into hydrogen peroxide, which is actually not a radical itself but generates the most reactive species, i.e., hydroxyl anion radical under the appropriate conditions. GSH-Px enzyme catalyzes the reduction of hydrogen peroxide into water by glutathione [4, 5]. Cadmium, naturally found in cigarette, decreases the bioavailability of selenium and acts antagonistically to zinc, a cofactor for the SOD [3].

Selenium (Se) is a trace element distributed in small amounts in the soil and food and has remarkable variability in regional distribution and bioavailability [10, 11]. Its major function is as a cofactor for the enzyme GSH-Px. It is therefore essential in removing free oxygen radicals from the body and preventing oxidative stress [12]. However, reports in the last decade have revealed that Se provides protection against free oxygen radical-induced cell damage [13, 14]. The proposed mechanisms are mainly through the function of seleno-dependent enzymes and selenoproteins [11]. Vitamin E is a major lipid-soluble nutritional antioxidant and can trap organic free radicals and protect proteins and lipids in biological membranes from oxidative damage [15]. Recent studies [2, 16–18, 19] reported that vitamin E, Se, and GSH-Px were decreased in blood and tissues of humans and animals by

cigarette smoke. Cigarette smoke contains metals, notably the ROS-inducing cadmium, which can be counteracted by selenium [2]. Hence, Se and vitamin E administrations may play important roles in the defense against cigarette smoke induced-ROS toxicity in blood of rats.

There is overwhelming evidence that cigarette smoke causes blood toxicity by increase in oxidative stress. However, effects of Se and vitamin E combination on cigarette smoke exposure-induced oxidative stress in blood of rats have never been studied. In the current study, we aimed to evaluate whether there would be a protective effect of Se and vitamin E on LP, enzymatic antioxidants, and lipid profile values in cigarette smoke-induced blood toxicity in rats.

## Materials and Methods

### Animals

Forty male Wistar albino rats weighing  $200 \pm 20$  g were used for the experimental procedures. Rats were allowed 1 week to acclimate to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments. The temperature was maintained at  $22 \pm 2^\circ\text{C}$ . A 12:12 h light/dark cycle was maintained, with lights on at 06:00, unless otherwise noted. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by Firat University.

### Experimental Design

Forty animals were randomly divided into four groups as follows:

- Group I: Control group ( $n=10$ ): Placebo (physiological saline) was supplemented to the first group.
- Group II: Cigarette smoke-exposed groups ( $n=10$ ): Rats in the groups were exposed to cigarette smoke.
- Group III: Cigarette smoke-exposed groups plus selenium ( $n=10$ ): Se (sodium selenit and 1.4 mg/kg/over day) was intraperitoneally (i.p.) administered to animals of this group.
- Group IV: Cigarette smoke-exposed groups plus selenium and vitamin E ( $n=10$ ): Se (sodium selenit at 1.4 mg/kg/over day) and vitamin E (dl- $\alpha$ -tocopherol acetate at 100 mg/kg/over day) was intraperitoneally (i.p.) administered to animals consisting the group.

All groups except the control were exposed to cigarette smoke (five cigarettes per day) starting at the third day of the experiment and continuing for 27 days by a specific simple device as described in a previous study [13].

### Anesthesia and Blood Collection and Preparation of Blood Samples

At the end of the experiments, rats were anaesthetized with ether. The blood (4–6 ml) was taken from the heart, using a sterile injector, into tubes, protected against light.

Blood samples were separated into plasma and erythrocytes by centrifugation at  $1,500\times g$  for 10 min at  $+4^{\circ}\text{C}$ . The erythrocyte samples were washed three times in cold isotonic saline (0.9%, v/w), then hemolyzed with a ninefold volume of phosphate buffer (50 mM, pH 7.4). After addition of butylhydroxytoluol ( $4\ \mu\text{l}$  per ml), hemolyzed erythrocytes and plasma samples were stored at  $-30^{\circ}\text{C}$  for  $<3$  months pending measurement of enzymatic activity and LP levels.

#### Lipid Peroxidation Level Determination

Lipid peroxidation levels in the plasma samples were measured with the thiobarbituric-acid reaction by the method of Placer et al. [20]. The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of LP in the plasma were expressed as nanomole per milliliter. Although the method is not specific for LP, measurement of thiobarbituric-acid reaction is an easy and reliable method, which is used as an indicator of LP and ROS activity in biological samples.

#### Glutathione Peroxidase, Superoxide Dismutase, and Protein Assay

GSH-Px activities in erythrocytes were measured spectrophotometrically at  $37^{\circ}\text{C}$  and 412 nm according to the method of Lawrence and Burk [21] as described in our previous study [22]. Total (Cu–Zn and Mn) SOD activity was determined according to the method of Sun et al. [23]. The principle of the method is based briefly on the inhibition of nitroblue tetrazolium reduction by the xanthine/xanthine oxidase system as a superoxide generator. Activity was expressed as units per milligram protein (IU/g hemoglobin). Hemoglobin values were determined according to the cyanmethemoglobin method of Cannon [24].

#### Biochemical Analyses

Triacylglycerol (TAG), total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) values were determined by routine kits using an autoanalyzer (Olympus AU 600, Tokyo, Japan).

#### Statistical Analysis

Statistical analysis was performed by using analysis of variance-SPSS statistical package (version 9.05, SPSS Inc. Chicago, IL, USA) for Windows. Least significant difference tests were used to determine the differences between the groups. The level of statistical difference at  $p<0.05$  was considered as significant.

## Results

The mean plasma lipid profiles in four groups are shown in Table 1. The results showed that the plasma TAG ( $p<0.05$ ) and TC ( $p<0.01$ ) levels were significantly higher in cigarette smoke group than in control. However, the plasma TC levels in the cigarette smoke+Se ( $p<0.05$ ) and cigarette smoke+Se+vitamin E ( $p<0.01$ ) groups and TAG levels in the cigarette

**Table 1** The Effects of Selenium and Vitamin E on Plasma Triglycerides, Cholesterol, and HDL-Cholesterol Concentrations in Rats Exposed to Cigarette Smoke (mean  $\pm$  SD,  $n=10$ )

Parameters	Control ( $n=10$ )	Cigarette smoke ( $n=10$ )	Cigarette smoke +Se ( $n=10$ )	Cigarette smoke+Se+VE ( $n=10$ )
Triglycerides (mg/dl)	59.14 $\pm$ 6.33	73.14 $\pm$ 11.03*	66.28 $\pm$ 11.02	57.57 $\pm$ 10.50***
Cholesterol (mg/dl)	62.28 $\pm$ 8.19	95.28 $\pm$ 7.43**	79.71 $\pm$ 8.23***	68.57 $\pm$ 4.90****
HDL-C (mg/dl)	19.28 $\pm$ 3.54	17.57 $\pm$ 2.22	22.71 $\pm$ 3.14	21.00 $\pm$ 2.16

\* $p<0.05$  vs control group; \*\* $p<0.01$  vs control group; \*\*\* $p<0.05$  vs smoke group; \*\*\*\* $p<0.01$  vs smoke group

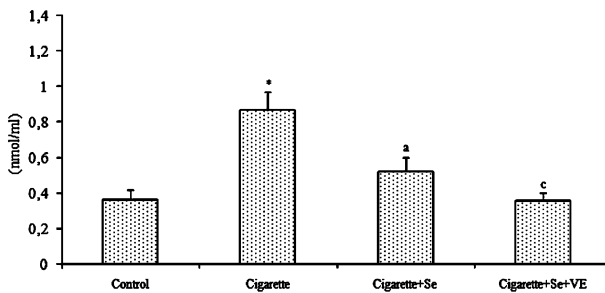
smoke+Se+vitamin E group ( $p<0.01$ ) were also significantly higher than in the cigarette smoke group.

The mean plasma LP values in four groups are shown in Fig. 1. The results showed that the plasma LP levels in the cigarette smoke group were significantly ( $p<0.05$ ) higher than in the control group. The LP levels in the Se ( $p<0.05$ ) and Se+vitamin E ( $p<0.001$ ) groups were significantly lower than in the cigarette smoke group.

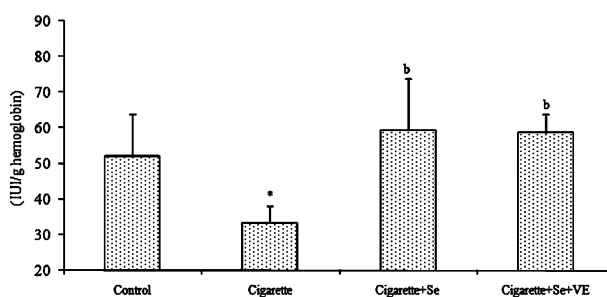
The mean GSH-Px and SOD activities in erythrocyte of four groups were shown in Figs. 2 and 3, respectively. The results showed that the erythrocytes GSH-Px ( $p<0.05$ ) and SOD ( $p<0.01$ ) activities in the cigarette smoke group were significantly lower than in the control group. However, the GSH-Px activities in the cigarette smoke+Se and the cigarette smoke+Se+VE groups ( $p<0.01$ ) and SOD activity in the cigarette smoke+Se+vitamin E group ( $p<0.05$ ) were significantly lower than in the cigarette smoke group. HDL-C levels were not changed by Se and vitamin E administrations.

## Discussion

In the current study, we observed that plasma LP, TAG, and TC levels were increased in rats exposed to cigarette smoke, whereas plasma erythrocyte GSH-Px and SOD activities were decreased in the rats exposed to cigarette smoke. Hence, cigarette smoke in the animals is characterized by increased oxidative stress levels. GSH-Px activity was increased by administrations of Se and VE, whereas LP levels were decreased by the Se and vitamin E treatments. To the best of our knowledge, the current study is the first to compare to action of the Se and VE combination with particular reference to its effects on oxidative stress and



**Fig. 1** The effects of selenium (Se) and vitamin E (VE) on plasma lipid peroxidation levels in rats exposed to cigarette smoke (mean $\pm$ SD,  $n=10$ ). \* $p<0.05$  vs control group; a  $p<0.05$ ; and c  $p<0.001$  vs cigarette group

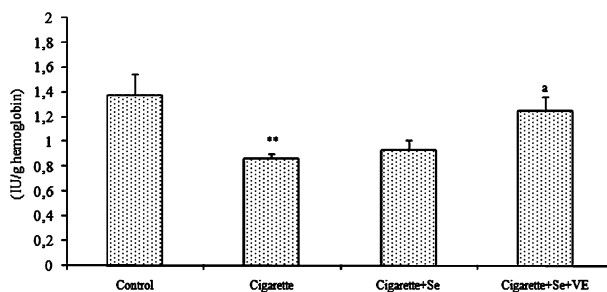


**Fig. 2** The effects of selenium (*Se*) and vitamin E (*VE*) on erythrocytes glutathione peroxidase activities in rats exposed to cigarette smoke (mean±SD,  $n=10$ ). \* $p<0.05$  vs control group;  $b p<0.01$  vs cigarette group

the antioxidant redox system using levels in cigarette smoke exposure-induced blood toxicity in rats.

Cigarette smoke contains large amounts of free radicals, pro-oxidants, and aldehydes which are toxic to tissue and blood cells. Exposure of body cells to cigarette smoke extracts leads to tissue oxidative stress by the increased production of ROS [25]. ROS could arise not only from the mainstream smoke of the cigarette smoke but secondly from endogenous sources, including uncoupled endothelial nitric oxide synthetase, NAD(P)H oxidase, xanthine oxidase, and the mitochondrial electron transport chain [5, 26]. These smoke-induced reactive substances cause extra- and intra-cellular antioxidants and may elicit oxidative damage to various cellular macromolecules [25, 26]. Smoking-elicited oxidative stress may enhance LP levels through the attack of the membrane lipids by ROS and organic free radicals. It is thus not surprising to find that cigarette smoke-exposed rats have increased levels of LP in plasma, which may be due to a decline in the activity and capacity of free radical scavengers and antioxidants in the blood circulation. In this study, we found that the cigarette smoke-induced decrease in erythrocyte SOD and GSH-Px activities were correlated with the increase in plasma level of LP in the rats exposed to cigarette smoke. This finding may suggest that SOD and GSH-Px are the major enzymatic antioxidants in the rats, which prevents plasma and erythrocyte lipid from oxidative damage caused by smoking-generated ROS and free radicals. This result is in accordance with previously published papers [16–19].

We observed an increase in antioxidant GSH-Px activity in the erythrocyte of Se and vitamin E-administered rats, suggesting antioxidant action of Se and vitamin E. LP contents were decreased in erythrocytes of rat exposed to cigarette smoke by Se and vitamin E treatments. Current study clearly shows that the Se and vitamin E treatments were able to



**Fig. 3** The effects of selenium (*Se*) and vitamin E (*VE*) on plasma superoxide dismutase activities in rats exposed to cigarette smoke (mean±SD,  $n=10$ ). \*\* $p<0.01$  vs control group;  $a p<0.05$  vs cigarette group

protect and increase not only the GSH-Px activity, indicative of a possible antioxidant effect, but also decrease LP levels. Chattopadhyay and Chattopadhyay [16] reported that supplementation of vitamin E antagonized the nicotine-induced effects though less effectively on LP, SOD, and GSH-Px values in protein-restricted condition of rats. In accordance with the results of current study, enhanced level of LP in nicotine treated rats has been shown to be accompanied by a significant decrease in the level of vitamin E, SOD, and GSH-Px [18, 19].

Inactivation of ROS can be carried out by the antioxidant enzymes [4, 5]. Superoxide anion is converted to hydrogen peroxide by SOD enzyme, and hydrogen peroxide is detoxified by GSH-Px and catalase [7, 11]. Activity of the enzymes in erythrocyte is considerable low, although it contains high iron contents in hemoglobin of erythrocytes. Therefore, the high iron contents in hemoglobin of erythrocytes and low antioxidant enzymes' activity of erythrocytes result in limited antioxidant defense in erythrocyte [8, 9]. In addition, cadmium, naturally found in cigarette, decreases the bioavailability of selenium, a cofactor for GSH-Px. Cadmium acts also antagonistically to zinc, a cofactor for the SOD [1, 12]. GSH-Px and SOD activities in the erythrocytes were lower in the cigarette smoke exposure group than in control, whereas GSH-Px activities in erythrocytes were increased in the Se and vitamin E treatment groups. The increased activities of GSH-Px could be due to its depletion or inhibition as a result of the increased production of free radicals. The increase in erythrocyte GSH-Px values in animals during vitamin E and Se treatments has been attributed to the inhibition of free radicals and LP [14, 17–19].

Consumption of Se and vitamin E has been associated with a reduced risk of cardiovascular disease, and antioxidants such as vitamin E and selenium can inhibit lipoprotein oxidation [1, 7]. Dietary consumption of vitamin E and Se results decrease in TC and triglycerides and in increased level of HDL-C in tobacco smokers [27]. Circulating concentrations of vitamin E, Se, and Se-dependent GSH-Px have been reported to be low in smokers [7, 16, 19]. The lack of correlation between circulating concentration of the antioxidants and their intake [27] suggests the possibility of their increased consumption by free radicals in cigarette smoke. This possibility is supported by *in vitro* findings: exposure of plasma to gas-phase cigarette smoke resulted in the destruction of vitamin E and Se-dependent GSH-Px enzyme [19, 28]. In accordance with results of current study, three studies reported that serum TC, phospholipids, and TAG were higher in the nicotine-treated rats than in controls, although HDL-C levels were lower in nicotine-treated rats than in control [16, 28, 29].

In conclusion, our blood results in rat exposure to cigarette smoke are consistent with a generalized enzymatic antioxidant abnormality in reports of blood and tissue in cigarette smoke animals and humans. However, the vitamin E and Se supplementation have protective effect on oxidative stress and antioxidant redox system in erythrocytes and plasma. The beneficial effect of vitamin E and Se on enzymatic antioxidant systems was regulation of GSH-Px and SOD activities and lipid profiles and LP levels in the blood. Measurement of plasma and erythrocytes LP, SOD, and GSH-Px values could be important diagnostic evaluations especially in cigarette smokers, and also, supplementation of Se and vitamin E in case of depletion seem to be useful. Hence, the results in blood may help physicians on the treatment of oxidative stress-dependent blood toxicity and lipid profile abnormalities in cigarette smokers by Se and vitamin E.

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