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The Investigation of the Antioxidative Properties of the Synthetic *Organoselenium* Compounds in Liver Tissue of Rat with Histological and Biochemical Analyses

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ABSTRACT

Background: Oxidative stress is described as the formation of toxic effect due to the deficiency of cellular antioxidative level toward the level of reactive oxygen species (ROS). The excess production of ROS or the decrease in the antioxidative defense system could be the cause for oxidative stress. 7,12-dimethylbenz[a]anthracene (DMBA) that is known to be the major cause the increment in lipid peroxidation level and the oxidative damage in the rat liver. As a fundamental trace elements, selenium as a part of anti-oxidative defense system is responsible for the immune system as part of enzymes in defense system.

Methods: Organoselenium compounds [1-isopropyl-3- methylbenzimidazole-2-selenone (Se I) and 1, 3-di-pmethoxybenzylpyrimidine- 2-selenone (Se II)] that were prepared in the laboratories. The effects of synthetic organoselenium compounds (Se I and Se II) against DMBA-induced changes in levels of some [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) activities and total glutathione (GSH), malonedialdehyde (MDA)] parameters in rat liver were investigated. Histopathological changes in the liver tissues of rats were examined by light microscopy.

Results: Because the selenium has an anti-oxidative properties toward the damaged induced cells, organoselenium compounds prepared in our laboratories, Se I and Se II, have tested for chemically induced rat liver tissues. The results showed that endogen antioxidant enzymatic activities changes and the preventing of oxidative damage in lipid peroxidation are important findings *in vivo* of this research.

Conclusion: Various changes were observed in liver tissue of rats in the all experimental groups.

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Introduction

Environmental sources of free radicals and reactive oxygen species (ROS) include some pollutants, organic solvents, pesticides, tobacco smoke (1). Polycyclic aromatic hydrocarbons (PAHs) are also an important source of environmental stress emitters, and their long-term bioaccumulation causes severe environmental pollution. 7,12-dimethylbenz[a]anthracene (DMBA) is a PAH known to cause tumors in rats. DMBA exposure causes a number of serious behavioral disfunctions and physiological disease processes, including cancer and aging (2). Oxygen-derived free radicals as highly reactive chemical species have importance in the aging process

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and they are also, either directly or indirectly, involved in various clinical disorders, such as atherosclerosis, reperfusion injury, cancer, etc. Because of the health problems induced by environmental pollutants, the antioxidant potential of selenium (Se) is the subject of intensive studies (3-5). Se is an essential micronutrient with well-known antioxidant characteristics. As a component of glutathione peroxidase, Se can scavange intracellular free radicals directly or indirectly. Pharmacologically, consumption of 200 µg Se per day by cancer patients reduces mortality and decreases the incidence of many diseases including liver, lung, colorectal and prostate cancers (6,7). Selenium-containing heterocycles are of increasing interest because of their chemical properties and biological activities. New approaches for the synthesis of selenium heterocycles by using more stable, less toxic, and easily accessible selenium reagents are of great interest. Finally, there has been a great deal of works carried out on selenium metabolism. Some inorganic or organic forms of selenium have been tested as possible cancer chemo-preventive agents. The data of these studies have been demonstrated to inhibit or delay the process of carcinogenesis induced by chemical carcinogens. In result, selenium-containing molecules may be better nucleophilic (and, therefore, antioxidants) than classical antioxidants has led to the synthesis of organoselenium compounds (1, 9-11). It is well known that cytotoxic factors in hepatocytes is involved among the other factors increasing ROS. The simultaneous increase of ROS levels could cause, inducing damage and reducing cell viability. ROS can be released from the hepatic vascular endothelium, platelets and Kupffer cells as a response to ischemia-reperfusion injury and circulatory shock. In response to tissue damage and inflammation induced by a variety of xenobiotics including acetaminophen, carbon tetrachloride, ethanol and endotoxin, as well as disease states such as viral hepatitis and postischemic and regenerative injury, the liver produces large quantities of ROS (11-13). In this work terms of the effects of organoselenium compounds, such as [1-isopropyl-3- methylbenzimidazole-2-selenone (SeI) and 1,3-di-pmethoxybenzylpyrimidine-2-selenone (SeII)], prepared (14) in laboratory were investigated in liver tissues of DMBA-induced rats with biochemical and histological analyses.

Materials and Methods

Animals and Experimental Design

In this study, thirty five healthy female albino Wistar rats (body weight 180-200 g, three to four months old) were divided into five groups, each consisting of eight animals. Animals were maintained under standardized light cycle conditions (12 h light:12 h dark period) at a temperature of 22±2 °C with free access to food (standard pellet chow diet) and water. The animals were obtained from the Experimental Animal Breeding and Research Center, Medicine School of Inonu University. Each rat was weighed just before the start of the study. All drugs were administered intraperitoneally (i.p.). DMBA was dissolved in corn oil and rats were injected with 50 mg/kg body weight. Se I and Se II were dissolved in corn oil and rats were injected with 25 µmol/kg. Rats in group I were used as control (pellet food and water were given). Animals in group II received only the vehicle solution, i.e. corn oil at two day intervals for four weeks. Rats in group III were given a single dose of 50 mg/kg DMBA and were sacrificed four weeks later. Animals in group IV received DMBA as in group III, but after 6 h of DMBA application Se I was administered at 25 µmol/kg at two day intervals for four weeks. Rats in group V were treated exactly as in group IV, except that Se II was used instead of Se I.

The ethical rules raised by "Guide for the Care and Use of Laboratory Animals" (15) were obeyed during this study which was closely scrutinized by the ethical commission of Medicine School of Inonu University.

Structure of synthetic organoselenium compounds

Se I and Se II (Figure 1. a and b) were synthesized in our laboratories by previously published methods [14]. The compounds synthesized were characterized by 1H NMR (300 MHz), 13C NMR (75.5 MHz), FTIR spectroscopic techniques and micro analysis.

Se I yield: 75%, mp: 96–97 °C. FT-IR, ν(C Se): 1407.9 cm^{-1}. 1H NMR (300 MHz, CDCl3), δ: 1.61 [d, J 7 Hz, 6H, CH(CH3)2]; 3.91 (s, 3H, N–CH3); 5.70 [sept., J 7 Hz, 1H, CH(CH3)2]; 7.24–7.76 (m, 4H, Ar–H). 13C NMR (75 MHz, CDCl3), δ: 20 and 33.5 [CH(CH3)2]; 51.6 (N–CH3); 109.7, 111.2, 122.8, 123, 131.2 and 134.2 (Ar–C); 165.8 (C Se). Anal. Calcd. For C11H14N2Se:C, 51.96;H, 5.55; N, 11.02; found: C, 51.98; H, 5.52;N, 11.04.

Se II yield: 80%, mp: 164–165 °C. FT-IR, ν(C Se): 1511 cm^{-1}. 1H NMR (300 MHz, CDCl3), δ: 1.74 (quin., J 5.9 Hz, 2H, CH2CH2CH2); 3.12 (t, J 5.9 Hz, 4H, CH2CH2CH2); 3.70 (s, 6H, CH2C6H4OCH3); 5.34 (s, 4H, CH2C6H4OCH3); 6.79 and 7.28 (d, J 8.6 Hz, 8H, CH2C6H4OCH3). 13C NMR (75 MHz, CDCl3), δ: 21.2 and 46 (CH2CH2CH2); 55.7 (CH2C6H4OCH3); 61.9 (CH2C6H4OCH3); 114.4, 129.4 and 129.5 (CH2C6H4OCH3); 159.6 (C Se).Anal. Calcd. For C20H24N2O2Se: C, 59.42;H, 6.37; N, 7.17; found: C, 59.40; H, 6.37; N, 7.16.

Preparation of tissues for biochemical analyses

All the animals were successively sacrificed after being anesthetized with 75 mg/kg of sodium pentobarbital. After the treatments, chests of rats were opened the vena cava was cut and 30 mL of 0.9% NaCl was injected into the heart to rinse blood from the body. The lung and kidney were removed and frozen in liquid nitrogen. Tissues were stored at -80°C until used. The tissues were separated into three parts for determination of enzymatic activity,

lipid peroxidation and histological analyses. The samples for enzyme analyses were homogenized in PBS buffer (pH 7.4) using PCV Kinematica Status Homogenizer. Homogenized samples were then sonicated for 1.5 min (30 s sonications interrupted with 30 s pause on ice). Samples were then centrifuged at 17,000 rpm for 15 min and supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at -80°C. The second part of tissues homogenate was used for lipid peroxidation analyses. Tissue was washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for MDA, the product of lipid peroxidation.

Protein assay

The protein content of the supernatants for enzyme assays and MDA assay was determined using the colorometric method of (16) using BSA as the standard. All analyses were performed in duplicates.

Enzyme assays

The samples were determined spectrophotometrically for CAT (mmol/mg protein), SOD (ng/mg protein), Se-GSH-Px (nmol/mg protein), GR (mmol/mgprotein) activities, and MDA (nmol/mg protein), GSH (nmol/mg protein) levels in the supernatant fraction.

CAT activity

CAT activity was measured at 37 °C by following the rate of disappearance of H_2O_2 at 240nm (17).

Cu, Zn–SOD activity

SOD activity in the supernatant fraction was measured using the xanthine oxidase/cytochrome c method (18), where one unit of activity is the amount of enzyme needed to cause halfmaximal inhibition of cytochrome c reduction.

Se-GSH-Px activity

Se-GSH-Px activity was determined in a coupled assay with glutathione reductase and by measuring the rate of NADPH oxidation at 340 nm using hydrogen peroxide as the substrate (19).

GR activity

Glutathione reductase is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to GSH. This assay is based on the oxidation of NADPH to NADP catalyzed by a limiting concentration of glutathione reductase. One GR activity unit is defined as the amount of enzyme catalyzing the reduction of 1 mmol of GSSG per minute at pH 7.6 and 25°C (20).

GSH assay

The formation of 5-thio-2-nitrobenzoate (TNB) is followed spectrophotometrically at 412 nm (21). The amount of GSH in the extract was determined as nmol/mg protein utilizing a commercial GSH as the standard.

Lipid peroxidation assay

The analysis of lipid peroxidation was carried out as described (22) with a minor modification. The reaction mixture was prepared by adding 1 mL homogenate into 4 mL reaction solution. MDA results were expressed as nmol/mg protein in the supernatant.

Preparation of tissue samples for histological measurement

After each application, both the experimental and control rats were sacrificed for histopathological examination. Liver tissues were transferred to 10% formaldehyde solution for 24 h for fixation. Following fixation, the tissues were washed for 24 h with running tap water in a beaker. The liver tissues were dehydrated in graded ethyl alcohol solutions. Tissues were cleared twice in xylene and embedded in paraffin. Sections (5−10 μm thick) were made and stained with haematoxylin and eosin, and all sections were examined under light microscopy. Three different fields of view per slide per rat haphazardly were selected and the results from each observation were combined for the final results.

Statistical analysis

The data were analyzed with SPSS 9.0 for Windows using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range test in which the significance level was defined as $P<0.05$.

Results

Biochemical findings

Changes in biochemical parameters such as CAT, SOD, Se-GSH-Px, GR activities, and GSH, MDA levels in rat liver tissues caused by application of synthetic organoselenium compounds (Se I and Se II) in DMBA induced rats are presented in Table 1.

CAT, SOD, Se-GSH-Px, GR activities in DMBA group statistically significantly decreased according to control group ($P<0.05$) (Table 1). CAT, SOD, Se-GSH-Px, GR activities in DMBA+Se I and DMBA+Se II groups significantly increased compared to DMBA group ($P<0.05$) (Table 1). GSH level in rat liver tissue of DMBA group did not change statistically significantly according to control group ($P>0.05$) (Table 1). There were statistically significantly increases GSH levels in DMBA+Se I and DMBA+Se II groups compared to data of control and DMBA groups ($P<0.05$) (Table 1). MDA level in DMBA group of rat liver tissue statistically significantly increased according to control group ($P<0.05$) (Table 1). There were statistically significantly

Table 1. Changes on liver CAT, SOD, GSH-Px, GR activities, and GSH, MDA levels with novel synthesized organoselenium compounds administration in DMBA-induced rat

Parameters / Groups	CAT (μmol/mgP)	SOD (ng/mgP)	GSH-Px (nmol/mgP)	GR (μmol/mgP)	GSH (nmol/mgP)	MDA (nmol/mgP)
Control	129.00±18^{a}	1.13±0.12^{a}	4.54±0.92^{a}	1.83±0.08^{c}	2.38±0.57^{b}	0.11±0.016^{b}
Corn Oil	83.33±2.29^{bc}	0.37±0.01^{d}	2.00±0.49^{b}	1.56±0.12^{cd}	3.52±0.27^{b}	0.13±0.009^{ab}
DMBA	78.48±6.43^{c}	0.53±0.03^{cd}	1.91±0.38^{b}	1.36±0.07^{d}	2.14±0.16^{b}	0.15±0.002^{a}
DMBA+Se I	115.00±9.72^{ab}	0.74±0.02^{b}	2.62±0.20^{ab}	2.79±0.15^{a}	8.34±1.28^{a}	0.12±0.004^{ab}
DMBA+Se II	96.98±4.08^{ab}	0.69±0.005^{bc}	4.16±1.18^{ab}	2.24±0.17^{b}	6.68±0.63^{a}	0.10±0.004^{b}
P	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

All data points are the average of n = 8 with ±S.D.
Different letters (a–d) indicate statistically significant (P<0.05).

decreases MDA levels in DMBA+Se I and DMBA+Se II groups compared to DMBA group (P<0.05) (Table 1).

Histopathological findings

The histopathology of rat liver is shown in figure 2.a,b,c,d,e,f. Histological observations in rat liver tissues of control, corn oil, DMBA, DMBA+SeI and DMBA+Se II groups were obtained.

When investigated liver tissue sections of control group which dyed by hematoxylin-eosin (HE), it was observed that polygonal hepatocytes that painted eosinophilic usually contain single nucleus and occasionally contain euchromatic nucleus and create hepatocyte cords that arranged radially in central vein (arrow) around. There were sinusoids in among these hepatocyte cords. Surrounded by connective tissue in the portal areas, usually each artery, vein and bile duct were observed. General histological appearance was consistent with normal liver histology (Figure 2.a). In liver tissue sections which belonging to corn oil application group, It was observed that hepatocytes are polygonal, contain single and double euchromatic nucleus and have normal view painted eosinophilic. Hepatocyte cords and sinusoids were arranged radially in around of vena centralis (arrow). General view was representing the classic liver lobule (Figure 2.b). When investigated liver tissue sections of DMBA group, it was observed that degeneration of hepatocytes (thick arrow) as large areas in the liver parenchyma and dilatation of sinusoid. In the parenchyma were observed rare lymphocytes (thin arrow) (Figure 2.c).

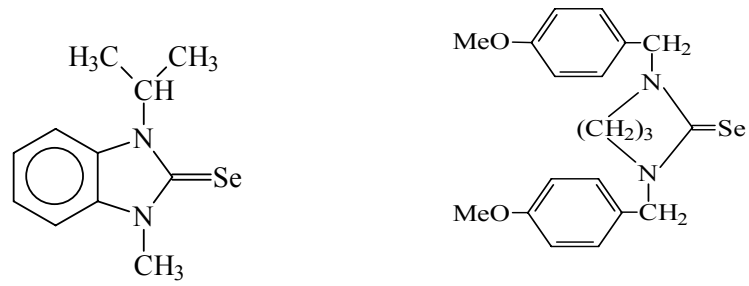
It was observed that dense lymphocytic infiltration in portal areas (aster) and occasionally decompose normal blood vessels and ducts in the portal ares (arrow) (Figure 2.d) . When investigated liver tissue sections of DMBA+Se I compound group, it was observed that hepatocytes were normal morphology. It was found that parenchymal structure and the portal areas analogously to control group include an artery (thick arrow), and ven (thin arrow), bile duct (short arrow) and lymph vessels (aster) (Figure 2.e).

Polygonal hepatocytes that painted eosinophilic and has round euchromatic nucleus showed normal histological view in liver tissue sections of DMBA+Se II compound group. Hepatocyte cords sequence and sinusoidal structures among them were radial and represented the characteristics of normal liver parenchyma (aster). However, dense bile duct proliferation in the portal area and occasionally lymphocyte infiltration in the portal area of slight took attention (arrow) (Figure 2.f).

Discussion

PAHs sustain in the environment for a long time with bioaccumulation environmental pollution and drastic biological effects. DMBA is a polycyclic aromatic hydrocarbon known to cause toxic effects. The toxic effect of DMBA causes oxidative stress in rats. Because of the health problems induced by many environmental pollutants, many efforts have been undertaken in evaluating the relative antioxidant potential of selenium compounds (10, 23). Liver of rat was selected on the basis of functional criteria that made its preferential targets for environmental pollution metabolism. Production of free radicals, which can be caused by environmental pollution plays an important role in damage and loss of function in tissues and organs. Lipid peroxidation, which results from the oxidative injury of saturated and unsaturated lipids, has been broadly used as a marker of the induction of oxidative damage in rats suffering from environmental stress induced by polycyclic aromatic hydrocarbons. In our study, CAT, SOD, Se-GSH-Px, GR activities decreased according to control group in the studied liver tissues after exposure to DMBA. These findings strongly suggest that DMBA caused generalized oxidative stress in the rat liver. Thus, the elevated production of ROS

(**Se I**) (**Se II**)



(a). 1-*izo*propil-3-metilbenzimidazol-2-selenon **(b)** 1,3-di-*p*- metoksibenzilpirimidin-2-selenon

Figure 1. (a) 1-Isopropyl-3-methylbenzimidazole-2-selenone (Se I) and (**b)** 1,3-di-p-ethoxybenzylpyrimidine-2-selenone (Se II**)**

may be due to DMBA toxicity, although other effects, such as enzymatic inhibition or genotoxic damage, may also occur (11). When ROS against natural the protective systems are overrun, exogenous antioxidative compounds must be delivered. Finally, the investigation for new antioxidants as potential drugs is an active field of medicinal chemistry (24). In this study, antioxidant enzymes activities such as CAT, SOD, Se-GSH-Px, GR in the liver decreased in DMBA-exposed group. Stress occurred by DMBA-decreased antioxidant enzymes activities. But, Se I and Se II caused increases in inhibited antioxidant enzymes activities in liver of DMBA-exposed rats. These results suggest that Se I and Se II may play an important role in maintaining antioxidants systems as antagonist substances to DMBA-induced decreases in activities of antioxidant enzymes. New approaches for the synthesis of selenium heterocycles by using more stable, less toxic, and easily accessible selenium reagents have a great interest. The data of this work show that synthetic organoselenium compounds can regulate DMBA-induced stress-related changes in rat liver (25). Organoselenium compounds can contribute to the antioxidative defense systems of rat tissues that have been shown in some studies by researchers (3, 10, 11, 25-30). As many carcinogens produce free radicals *in vivo*, selenium compounds can act as a trap of free oxygen radicals and exerts its effect by scavenging free radicals and converting them into stable compounds. Oxidative factors may markedly increase the oxidative cell damage. Adequate antioxidant defense systems including micronutrient intake may prevent lipid peroxidation. Selenium has antioxidant properties and is scavengers of free radicals, thus preventing damage to tissues (31, 32). We can say that DMBA exposuring induces increase of ROS levels in the rat liver. This increased ROS production plays role in DMBA-induced liver damage. Se I and Se II differ in chemical structure, they showed similar chemopreventive effects on all cell types in the liver including hepatocytes, kupffer cells, stellate cells, and endothelial cells that have the capacity to generate ROS in terms of biochemical properties.

Epidemiological works have implied the protective and preventive effects of organoselenium compounds against a various cancers and their ability to reduce oxidative injury, enhance exert anticancer properties (31, 32).

Some hypotheses have been declared to explain the inhibition of tumorigenesis by supplemental selenium, including: protection against oxidative injury involving the selenium as an essential component of antioxidant enzymes, alterations in carcinogen metabolism, effect on the immune system (33). The study showed that selenium applied in combination with DMBA minimized its hazards. Se I and Se II both provide the antioxidant effects against DMBA-induced oxidative stress in rat liver. The data demonstrate a direct evidence for the preservation role of synthetic organoselenium compounds on the antioxidative defense system against DMBA as known PAH toxicity. These results might be related to the fact that PAHs are detoxicated by selenium, which thus enabled rat exposed to organic pollutants entering the environment to survive (2). Treatments of SeI and SeII were increased antioxidant enzymes activities and GSH levels. Also, the novel organoselenium compounds proved to be useful in decreasing the level of MDA in liver. Se I and Se II provided protection against lipid peroxidation

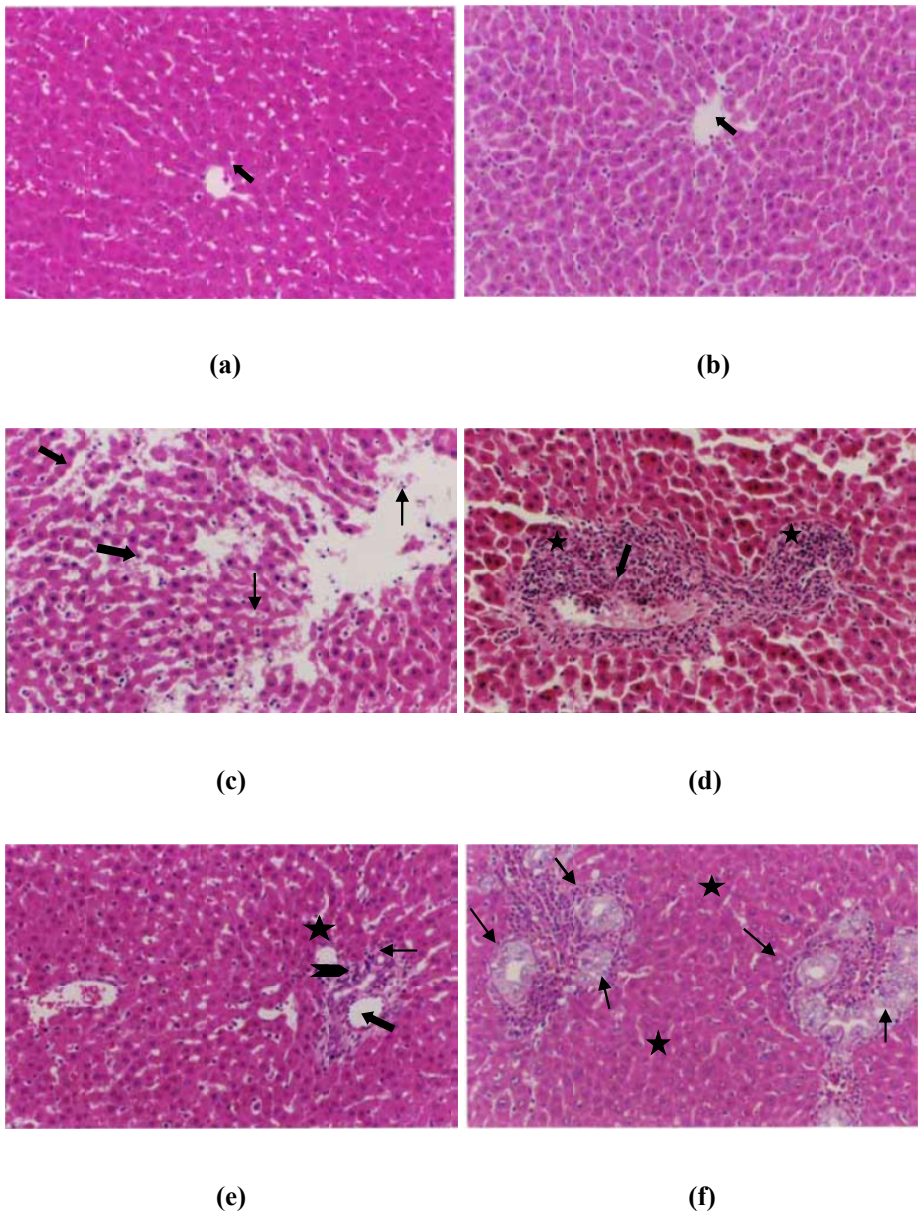


Figure 2. The histopathology of rat liver in control, corn oil, DMBA, DMBA+SeI and DMBA+Se II groups. All images: H&E stain
(a) Liver histological structure of control rat; normal hepatocytes (H-E x 20)
(b) with corn oil; normal histological structure (H-E x 20)
(c) Liver section of rat exposed only with DMBA, showing degeneration of hepatocytes and dilatation in sinusoids, lymphocytes in the liver parenchyma (H-E x 20)
(d) Liver section of rat exposed only with DMBA; dense lymphocytic infiltration and decomposes blood vessels and ducts in the portal ares (H-E x 20)
(e) Liver section of rat applied DMBA+SeI; normal histological structure including an artery, ven, bile duct and lymph vessels in parenchymal structure (H-E x 20)
(f) Liver section of rat applied DMBA+SeII; normal liver parenchyma and dense bile duct proliferation and occasionally lymphocyte infiltration in the portal areas (H-E x 20)

measured as MDA in liver. Both SeI and SeII provide chemoprevention against DMBA-induced oxidative stress in rat liver. The results of the present study parallel those found in the literature (3, 11, 26-30, 34, 35). Selenium can contribute to the antioxidative defense system of rats in DMBA-induced oxidative stress. This tolerance can be explained by the cofactor nature of selenium for Se-GSH-Px. The these data suggest that PAHs are capable of inducing biochemical parameters in rats that may cause physiometabolic dysfunction. In conclution, the study provide direct evidence for the chemopreventative role of selenium on the antioxidative defense system against the toxicity of organic pollutants.

In addition to, using diets rich in selenium could be beneficial in alleviating DMBA toxicity. It has been explained that selenium had inhibited cancer and chromosome damage as well as increased resistance to viral and bacterial infections (13, 36). The data of this study are consistent with the anticarcinogenic and free radical scavenging properties of organoselenium compounds demonstrated.

As a result, we conclude that DMBA treatment induces an increase in oxidative damage in rat tissues. This increase in oxidative production plays a role in DMBA induced liver damage. And as a result, it can be concluded that novel organoselenium compounds (SeI and SeII) provide a decrease in oxidative stress caused by DMBA induction in rat liver.

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References

1. Das RK, Ghosh S, Sengupta A, Das S, Bhattacharya S. Inhibition of DMBA/croton oil-induced two-stage mouse skin carcinogenesis by selenocyanate. Eur J Cancer Prev 2004; 13(5): 411-7.
2. Demir I, Demirbag Z. Biodegradation of polycyclic aromatic hydrocarbons. Tr J Biol 1999; 23: 293-302.
3. Talas ZS, Ozdemir I, Yilmaz I, Gok Y, Orun I. The investigation of the antioxidative properties of the novel synthetic organoselenium compounds in some rat tissues. Exp Biol Med 2008; 233: 575-9.
4. Ghosh P, Singha Roy S, Basu A, Bhattacharjee A, Bhattacharya S. Sensitization of cisplatin therapy by a naphthalimide based organoselenium compound through modulation of antioxidant enzymes and p53 mediated apoptosis. Free Radic Res 2015; 49(4):1-19.
5. Singha Roy S, Chakraborty P, Ghosh P, Ghosh S, Biswas J, Bhattacharya S. Influence of novel naphthalimide-based organoselenium on genotoxicity induced by an alkylating agent: the role of reactive oxygen species and selenoenzymes. Redox Rep 2012;17(4):157-66.
6. Pagmantidis V, Meplan C, van Schothorst EM, Keijer J, Hesketh JE. Supplementation of healthy volunteers with nutritionally relevant amounts of selenium increases the expression of lymphocyte protein biosynthesis genes. Am J Clin Nutr 2008; 87: 181-9.
7. El-Bayoumy K, Sinha R. Mechanisms of mammary cancer chemoprevention by organoselenium compounds. Mutat Res 2004; 551:181-97.
8. Garud DR, Koketsu M, Hideharu I. Isoselenocyanates: a powerful tool for the synthesis of selenium-containing heterocycles. Molecules 2007; 12: 504-35.
9. Sieja K, Talerczyk M. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol Oncol 2004; 93: 320-7.
10. Talas ZS, Ozdemir I, Gok Y, Ates B, Yilmaz I. Role of selenium compounds on tyrosine hydroxylase activity, adrenomedullin and total RNA levels in hearts of rats. Regul Pept 2010; 159: 137-41.
11. Talas ZS, Bayraktar N, Ozdemir I, Gok Y, Yilmaz I. The effects of synthetic organoselenium compounds on nitric oxide levels in DMBA- induced rat liver. J Environ Biol 2009; 30(4): 591-3.
12. Laskin JD, Heck DE, Gardner CR, Laskin DL. Prooxidant and antioxidant functions of nitric oxide in liver toxicity. Biotechnology 2001; 3: 261-71.
13. Nagar S, Zalator JJ, Blanchard RL. Human UGT1A6 pharmacogenetics: Identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes in human liver tissue and in cultured cells. Pharmacogenetics 2004; 14: 487-99.
14. Talas ZS, Gok Y, Ozdemir I, Ates B, Gunal S, Yilmaz I. Synthesis, antioxidant and antimicrobial properties of two organoselenium compounds. Pak J Pharm Sci 2015; 28(2): 611-16.
15. Washington DC. Guide for the care and use of laboratory animals. National Academy Press, 1996.
16. Lowry O, Rosebrough NJ, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. J Biol Chem 1951; 193:265–75.
17. Luck H. Methods of Enzymatic Analysis. Verlag Chemie, Academic Press, New York, (1963) pp.885–8.
18. McCord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-55.
19. Lawrance RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976; 71: 952-8.
20. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985; 11: 484-490.
21. Theodorus P, Akerboom M, Sies H. Assay of glutathione, glutathione disulfide and glutathione mixed disulfides in biological samples. Methods Enzymol 1981; 77: 373-83.
22. Beuge JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978; 52: 302-10.
23. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J Trace Elem Med Biol 2004; 18: 113-21.
24. Shadab GG, Ahmad ME, Azfer MA. Anticlastogenic action of vitamin C against genotoxicity of estrogenic drug diethylstilbestrol (DES) in the human lymphocyte chromosomes. J Environ Biol 2006; 27: 85-88.
25. Ozdemir I, Talas ZS, Gok Y, Ates B, Yilmaz I. Changes in tyrosine hydroxylase activity, adrenomedullin and total RNA levels by treatment of organoselenium compounds in rat hypothalamus exposed to DMBA. Fresen Environ Bull 2010; 19(4a): 664-8.
26. Talas ZS, Ozdemir I, Yilmaz I, Gok Y. Antioxidative effects of novel synthetic organoselenium compound in rat lung and kidney. Ecotox Environ Safe 2009; 72(3): 916-21.
27. Talas ZS, Yilmaz I, Ozdemir I, Ates B, Gok Y, Cetinkaya B. Role of synthesised organoselenium compounds on protection of rat erythrocytes from DMBA-induced oxidative stress. Biol Trace Elem Res 2009; 128: 167-75.
28. Ozdemir I, Talas ZS, Gul M, et al. Inhibition of DMBA induced rat mammary duct damage by novel synthetic organoselenium compounds. Exp Anim 2006; 55(5): 449-55.
29. Selamoglu Z, Ozdemir I, Ates B, Gok Y, Orun I, Yilmaz I. The effects of novel synthetic organoselenium compounds on hematological parameters in DMBA-induced rats. Fresen Environ Bull 2007; 16(6): 596-600.
30. Ozdemir I, Selamoglu Z, Ates B, Gok Y, Yilmaz I. Modulation of DMBA-induced biochemical changes by organoselenium compounds in blood of rats. Indian J Biochem Biophys 2007; 44(4): 257-9.
31. Rayman MP. The importance of selenium to human health. Lancet 2000;

356: 233-41.
32. El Bayoumy K. The protective role of selenium on genetic damage and on cancer. Mutat Res 2001; 475: 123-39.
33. Duffield-Lillico AJ, Reid ME, Turnbull BW,et al. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the nutritional prevention of cancer trial. Cancer Epid Biomarkers Prev 2000; 11: 630-9.
34. Basu A, Bhattacharjee A, Singha Roy S, et al. Vanadium as a chemoprotectant: effect of vanadium(III)-L-cysteine complex against cyclophosphamide-induced hepatotoxicity and genotoxicity in Swiss albino mice. Eur J Biochem 2014; 19(6): 1141-6.
35. Bhattacharjee A, Basu A, Ghosh P, Biswas J, Bhattacharya S. Protective effect of Selenium nanoparticle against cyclophosphamide induced hepatotoxicity and genotoxicity in Swiss albino mice. J Biomater Appl 2014; 29(2): 303-17.
36. Carlo WF, Hummer AJ, Schwartz L, et al. Extent of hepatic resection does not correlate with toxicity following adjuvant chemotherapy. J Surg Oncol 2004; 87: 85-90.