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The effects of synthetic organoselenium compounds on nitric oxide in DMBA - induced rat liver

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Abstract: *DMBA (7,12-dimethylbenz[a]anthracene) is known to generate DNA-reactive species during their metabolism, which may enhance oxidative stress in cells. Since selenium is known as a non-enzymic antioxidant, health problems induced by many environmental pollutants, have stimulated the evaluation of relative antioxidant potential of selenium and synthetic organoselenium compounds. Therefore, we aimed to evaluate chemopreventive potential of synthetic organoselenium compounds by monitoring level of liver nitric oxide. In this study, adult female Wistar rats were treated with DMBA and the novel organoselenium compounds (Se I) and (Se II) in the determined doses. DMBA-induced in rats, the effects of organoselenium compounds on nitric oxide levels in rat liver was studied. In this study, it has been observed a statistically significant increase in (Nitric Oxide) levels for the liver of rat exposed to DMBA ($p<0.05$). However with administration of Se I and Se II there was a statistically significant decrease in NO levels ($p<0.05$). The ability of the organoselenium compounds to prevent oxidative damage induced by DMBA in rat livers was rationalized. Protection against nitric oxide measured in Se I and Se II treated groups were provided by synthesized organoselenium compounds. Se I and Se II both provided chemoprevention against DMBA-induced oxidative stress in rat liver.*

Key words: *DMBA, Liver, Nitric oxide, Rat and synthetic organoselenium compounds*
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Introduction

DMBA (7,12-dimethylbenz[a]anthracene) is a polycyclic aromatic hydrocarbon (PAH) known as cause tumors in rats (Desai *et al*., 2001). PAHs, such as petroleum and petroleum derivatives, are widespread organic pollutans entering the environment, through oil spills and incomplete combustion of fossil fuels. Since most PAHs are persist in the environment for a long period of time bioaccumulation occur which causes environmental pollution and effects biological equilibrium drastically (Demir and Demirbag, 1999; Jagadeesan and Pillai, 2007). The toxic effect of DMBA causes a stress in rats (Sun, 2001).

Antioxidant defense molecules fall into one of two categories: enzymatic or nonenzymatic. Selenium is an essential element with physiological nonenzymatic antioxidant properties (Nogueira *et al.,* 2003). Selenium counteracts cancer and chromosome damage as well as increases our resistance to viral and bacterial infections (Nagar *et al.,* 2004; Carlo *et al.,* 2004). When the natural protective systems against reactive oxygen species are overrun, exogenous antioxidative compounds must be delivered (Shadab *et al.,* 2006). Consequently, the search for new antioxidants as potential drugs is an active field of medicinal chemistry (Devillers *et al.,* 2001).

It is well known that cytotoxic factors, such as lipopolysaccharides, derange nitrogen metabolism in hepatocytes and nitric oxide (NO) is involved among the other factors regulating this metabolic pathway. The simultaneous increase of NO and reactive oxygen species (ROS) levels could cause peroxynitrite synthesis, inducing damage and reducing cell viability (Tabuchi *et al.,* 2000). Nitric oxide (NO) can be released from the hepatic vascular endothelium, platelets and Kupffer cells as a response to ischemia-reperfusion injury and circulatory shock (Laskin *et al.,* 2001). 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 nitric oxide (Laskin *et al.,* 2001).

In this study DMBA-induced rats were studied in terms of the effects of organoselenium compounds, such as [1-isopropyl-3-methylbenzimidazole-2-selenone (Se I) and 1, 3-di-*p*-methoxybenzylpyrimidine-2-selenone (Se II)], prepared (Aygun *et al.,* 2003; Gok *et al.,* 2004) in laboratory were studied.

Materials and Methods

Setting of groups: In the study, thirty five healthy female Albino Wistar rats (body weight 150-200 g) were divided into five groups, each consisting of six to seven animals. Each rat was weighed just before the start of the study. All drugs were administered intra-peritoneal (i.p.). DMBA was dissolved in corn oil and rats were i.p.

injected 50 mg kg^{-1} body weight. Organoselenium compounds (Se I and Se II) were dissolved in corn oil and rats were injected 25 µmol kg^{-1}. The structure of organoselenium compounds Se I and Se II is given as Fig. 1A and 1B.

Animals were divided into five groups. Animals in the Group I were used as a control. Animals in the group II received only the vehicle solution, corn oil for four weeks at two days intervals. Animals in the Group III were given a single dose of 50 mg kg^{-1} DMBA and were sacrificed four weeks later. Rats in the Group IV also received DMBA as in Group III, but after 6 hr of DMBA application the Se I compound of 25 µmol kg^{-1} body weight was applied for four weeks at two days intervals. Animals in the Group V were also treated exactly as Group IV animals, except that Se II compound was used instead of Se I. All the animals were successively sacrificed after anaesthetized with 75 mg kg^{-1} of sodium pentobarbital.

Sample preparation: 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 liver were removed and frozen in liquid nitrogen at -80°C until used. Liver tissue NO levels were measured by a modification of the Griess reaction (Cortas and Wakid, 1990; Navarro-Gonzalves *et al.,* 1998).

Statistical analysis: Total nitrite in the liver tissue data was 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 defined as $p<0.05$.

Results and Discussion

In this study, it has been observed a statistically significant increase in NO levels for the liver of rat exposed to DMBA ($p<0.05$) However with administration of organoselenium compounds (Se I and Se II) there was a statistically significant decrease in NO levels ($p<0.05$) (Table 1). The antioxidant activity of Se I and Se II compounds on free radical induced NO levels has been decreased. Therefore, novel synthetic organoselenium compounds inhibited the development of DMBA-induced oxidative stress in rat liver.

The data show that the increased NO production plays a role in liver damage induction, that follows DMBA-induced oxidative stress. The hepatocellular injury attributed to NO may be due either to its direct cytotoxicity or its reaction with superoxide to produce the toxic nitrogen metabolite peroxynitrite (Yamamoto *et al.,* 2000).

The results of the present study are consistent with the anticarcinogenic and free radical scavenging properties of organoselenium compounds reported. Epidemiological studies have documented the protective effects of organoselenium compounds against a wide variety of cancers and their ability to reduce oxidative damage, enhance exert anticancer effects (Rayman, 2000; El-Bayoumy, 2001). Administration of organoselenium compounds for 4 weeks reduced the incidence of DMBA induced oxidative damage. Our findings demonstrate that

Table - 1: Levels of total nitrite in the liver with Se I and Se II compounds administration in DMBA induced rats

Groups	Total nitrite (µ mol g^{-1} tissue)
Control	25.42 ± 1.41^{d}
Corn oil	42.50 ± 1.47^{c}
DMBA	81.25 ± 1.17^{a}
DMBA+Se I	72.04 ± 1.72^{b}
DMBA+Se II	71.20 ± 1.06^{b}

All data points are the average of n=7 with ± SD. ^{abc} statistically significant ($p< 0.05$)

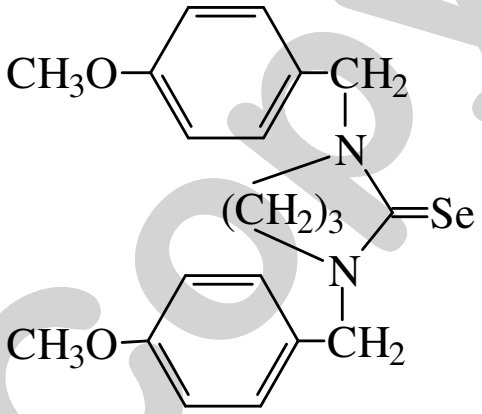

Fig. 1A: 1-*iso*propyl-3-methylbenzimidazole-2-selenone (Se I)

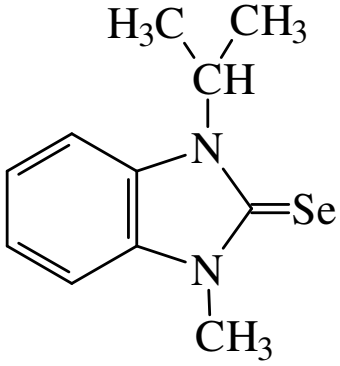

Fig. 1B: 1,3-di-*p*-methoxybenzylpyrimidine-2-selenone (Se II)

organoselenium compounds exerts its antioxidant effects by increased NO levels, in the rat liver.

Organoselenium compounds (Se I and Se II) provided protection against increased NO in DMBA treated groups. Se I and Se II both provide chemoprevention against 7,12-DMBA-induced oxidative stress in the rat liver. The results of studies carried out in rat blood serum provide a direct evidence for the preservation role of synthetic organoselenium compounds on the rat liver and blood AST (Aspartate Aminotransferase=SGOT; Serum Glutamic-Oxaloacetic Transaminase), ALT (Alanine transaminase=SGPT; Serum Glutamate Pyruvate Transaminase) activities against DMBA as known polycyclic aromatic hydrocarbon (PAH) toxicity (Ozdemir

et al., 2007). This might be related to the facts that PAH are detoxicated by selenium, which thus enabled rat exposed to organic pollutants entering the environment to survive. The results of this study are parallel with literature (Sugie *et al.,* 2000).

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. Adequate antioxidant defense systems including micronutrient intake may prevent lipid peroxidation. Oxidative factors may markedly increase the oxidative cell injury (Singh and Rana, 2007*;* Flora *et al*., 2007) Selenium has antioxidant properties and is scavengers of free radicals, thus preventing damage to tissues (Sieja and Talerczyk, 2004). In summary, we conclude that DMBA treatment induces an increase of NO levels in the rat liver. This increased NO production plays role in DMBA-induced liver damage. The results found herein was found to be the most promising because, both of novel organoselenium compounds have effect of decrease in oxidative stress made by DMBA induction ($p<0.05$). We report the chemopreventive potential of Se I and Se II againts levels of NO in liver. Though 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 nitric oxide in terms of biochemical properties.

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