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EFFECTS OF SODIUM SELENITE ADMINISTRATION DURING DIETHYLNITROSAMINE INTOXICATION IN RATS

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ABSTRACT. The aim of this study was to evaluate the toxicity of a single dose of 100 mg/kg body weight of NDEA and to compare the effects of selenium administration before and after the NDEA dose (pre- vs. post-treatment), on the evolution of some biochemical parameters. Male Wistar rats were divided into five groups (n=5): control group, NDEA group (NDEA: 100 mg/kg bw by gavage, single dose), NDEA + Se3 group (NDEA: 100 mg/kg bw by gavage, single dose + Se^{+4}: 3 mg/kg bw by gavage, in the first four days), Se group (Se^{+4}: 3 mg/kg bw by gavage, for four days) and Se3 + NDEA group (Se^{+4}: 3 mg/kg bw by gavage, for four days before NDEA administration + NDEA: 100 mg/kg bw by gavage, single dose). The animals were sacrificed after ten days from NDEA administration and blood was collected. The biochemical parameters were determined using a RX Imola automatic analyzer. In the NDEA group uric acid, total bilirubin, direct bilirubin, HDL-cholesterol, LDL-cholesterol levels and ALT and AST activities were significantly increased compared to the control group. Total bilirubin, direct bilirubin, HDL-cholesterol and iron blood levels, as well as GGT and AST activities were significantly increased in NDEA+Se group, compared to the Se+NDEA group, whereas LDL-cholesterol levels and ALT activities were significantly increased in Se+NDEA group, compared to the NDEA+Se group. At this dose and observational period, NDEA was slightly toxic. The post-treatment with sodium selenite increased NDEA toxicity and was more severe than the pre-treatment.

Keywords: selenium, sodium selenite, diethylnitrosamine, biochemical parameters, rats.

INTRODUCTION

Nitrosamines are considered one of the most important classes of carcinogens, therefore posig a significant threat for human health (Aiub et al., 2003), and have been reported to occur in various foodstuffs (cheese, cured or cooked meat products, bacon, some bevarages) (Lijinsky, 1999 ; Levallois et al., 2000), in tobacco smoke and latex products (Altkofer et al., 2005). Moreover, nitrosamines may be formed in the human as a result of the reaction of between the nitrite ion and secondary and tertiary amines at a low gastric pH (Lijinsky, 1999; Ohsawa et al., 2003). Nitrites are formed by reduction of nitrates, which are often used to preserve various types of food, especially meat products. Nitrites used in the meat industry help to develop flavor, stabilize the red colour in meat products and have antioxidant effects (Hord et al., 2009). Diethylnitrosamine (N-nitrosodiethylamine, NDEA), an N-nitroso alkyl compound, is known for its hepatotoxic, carcinogenic and mutagenic potential to cause tumors in the gastrointestinal tract, liver, skin and other organs (Verna et al., 1996). At low doses (10 mg/kg body weight) NDEA causes hepatic fibrosis (Kim et al., 2005), while at higher doses NDEA inflicts serious damage, thus its toxicity is proportional with the dose (Verna et al., 1996). In rats, at weekly oral doses of 100 mg/kg body weight, NDEA causes severe hepatic cirrhoses characterized by large tubercles, death occurring after seven to fifteen weeks from the first dose (Steinhoff D, 1975). Despite the fact that N-nitroso compounds have been shown to be carcinogenic in animals (Aiub et al., 2003), little evidence has been found in the case of humans. NDEA is also known to cause oxidative stress and act as a mutagen after its activation by microsomal enzymes such as the cytochrome P450 enzymes (Bansal et al., 2005; Aiub et al., 2006). Other studies shown that exposure to this toxic increases lipid peroxidation and inhibits the antioxidant activity of glutathione peroxidase, superoxide catalase and dismutase (Banakar et al., 2004). Because oxidative stress plays an important role in NDEA toxicity, the use of some antioxidants, such as selenium, may be benefic in reducing its toxicity.

Selenium is an essential microelement, with multiple physiological roles, naturally found in soils. The inorganic forms of selenium with biological importance are selenate (SeO_4^{2-}) which is absorbed in high percentage but immediately excreted through urine and selenite (SeO_3^{2-}) which is absorbed in smaller percentage than selenate, but is better retained (Thomson et al., 1986). In animals, the most quantity of selenium is present as selenomethionine or selenocysteine. It has been shown that selenium can reduce the extent of liver cancer on induced hepatocarcinogenesis with NDEA in rats (Alwahaibi et al., 2010). Although various studies have shown that selenium possesses chemopreventive potential and has protective effect against oxidative damage (Raich et al., 2001), the mechanisms are not yet fully elucidated; however, some explanations have been given: the antioxidant action, the inhibition of angiogenesis and tumor cell invasion (Zeng et al., 2008).

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Thirunavukkarasu C et al. proved the beneficial effect of selenium administration (as sodium selenite) in NDEA intoxicated rats (Thirunavukkarasu C et al., 2003). It was proved that selenium-enriched malt had a better chemopreventive efficiency in decreasing the number of hepatoma nodules, relative liver weight and certain biochemical parameters in rats exposed to NDEA, than sodium selenite (Liu JG et al, 2006). The results obtained in another recent study demonstrated that beside the well known chemopreventive and chemotherapeutic role of selenite, cellular DNA damage is decreased or even prevented in various pathological conditions (Thirunavukkarasu C et al., 2008).

The aim of this study was to evaluate the toxicity of a single dose of 100 mg/kg body weight of NDEA and to compare the effects of selenium administration (as sodium selenite, for the same period of time and in the same dose) before and after the NDEA dose (pre- vs. post-treatment), on the evolution of some biochemical parameters.

MATERIALS AND METHODS

Chemicals and reagents

Sodium selenite and diethylnitrosamine (N-nitrosodiethylamine, NDEA) were purchased from Sigma Aldrich and were of analytical grade. A Se^{+4} 0,6 mg/ml and a NDEA 25 mg/ml solution were prepared using purified water, in order to be administered to rats. All other chemicals and materials used were purchased from commercial available sources.

Animals

A number of 25 male Wistar rats, aged 8 months were obtained from the animal housing facility of the University of Medicine and Pharmacy "Gr. T. Popa" Iaşi. The initial source of the animals was the Laboratory animal centre of the Cantacuzino Institute, Bucharest, Romania.

Experimental protocol

The committee for research ethics of the University of Medicine and Pharmacy "Gr. T. Popa" Iaşi approved the design of the experiments.

The rats went through a 7-11 day period of acclimatization before the start of the experiment. During the acclimatization and the experimental period the rats were given food (commercial standardized rat food) and water, *ad libitum* and were housed in collective cages, with the floor covered with sawdust, in relative constant environmental conditions (temperature 18-25° C). The light/dark cycle was approximately 14/10 hours. After the acclimatization period, the animals were weighed (initial weight 325-450 g), randomly divided in 5 groups (n = 5), the animals from each group being relocated in the same collective cage. The vehicle and the Se^{+4} and NDEA solutions were administered by gavage. The groups of animals, corresponding to different treatments, were: control group (negative control; vehicle (purified water) was administered only on days 1-4), NDEA group (positive control, NDEA: 100 mg/kg bw, single dose on day 1), Se group (therapeutic control, Se^{+4} as sodium selenite: 3 mg/kg bw, on days 1-4), Se + NDEA group (Se^{+4}, as sodium selenite: 3 mg/kg bw, for 4 days prior to NDEA administration (days -4 to -1) and NDEA: 100 mg/kg bw, single dose on day 1) and NDEA + Se group (NDEA: 100 mg/kg bw, single dose on day 1 and Se^{+4} as sodium selenite: 3 mg/kg bw, on days 1-4). On day 11 the animals were anaesthetized and blood was collected in by heart puncture. Euthanasia was a result of exsanguinations and heart damage.

Blood analysis

Blood was collected in vacutainer-type tubes without coagulant. The collected blood samples were prepared by centrifugation and other types of processes. The biochemical analyses were performed on an RX Imola® biochemistry analyzer. ELITech® kits were used for all the determined parameters. The fallowing biochemical parameters were determined, in order to assess certain pathological modifications:

- In order to assess liver cell integrity, by indirectly evaluating the permeability of the hepatocyte membrane, the enzymatic activities of alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST), as well as iron blood levels were determined.
- For assessing the liver activities, blood levels of urea, total bilirubin, direct bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol and gamma-glutamyl transferase (GGT) enzymatic activities were determined.
- For evaluating the renal function, urea, creatinine and uric acid blood levels were determined.

Statistical analysis

Statistical evaluation of the data was performed using one-way ANOVA followed by Tukey test. The statistical software used were Stats Direct, version 2.7 and Microsoft Excel 2007. Values were considered statistically significant for $P < 0,05$. Data calculated for each group are expressed as means ± standard error of the mean (SE).

RESULTS AND DISCUSSION

No mortality was observed in any group. Relatively low weight losses were observed in the NDEA + Se, Se and Se + NDEA groups (6,6 ± 2,32 %, 5,46 ± 2,34 % and 3,15 ± 1,86 % medium decreases in body weights of the rats at the end of the experimental period, compared to initial body weights). Non-significant increases in body weights of the rats from the control and NDEA groups were noticed.

Renal function

The parameters used for assessing the renal function were creatinine and urea and secondarily uric acid, which can also be an indicator for other types of metabolic disorders. No significant differences were noticed in urea blood levels from the NDEA and NDEA+Se groups, compared to the control group. Urea levels were higher in case of pre-treatment with selenium (Se+NDEA), compared to post-treatment (NDEA+Se) (Figure 1). Selenium, as single treatment (Se group), produced a slight increase in urea blood levels, compared to the control. At a different dose and dosing regimen, sodium selenite administration also produced an increase in urea blood levels (El-Demerdash FM, 2004).

No significant differences in creatinine blood levels were noticed between groups, with the exception of Se group, in which case apparently a slight increase was noticed, compared to control (Figure 2).

Significant higher uric acid levels were noticed in the NDEA and NDEA+Se groups, compared to the control group. Pre-treatment with selenium (Se+NDEA) led to a decrease in uric acid blood levels, compared with post-treatment (NDEA+Se) (Figure 3). The highest uric acid level was noticed in the Se group.

In none of the treatment groups severe impairment on the renal function was observed.

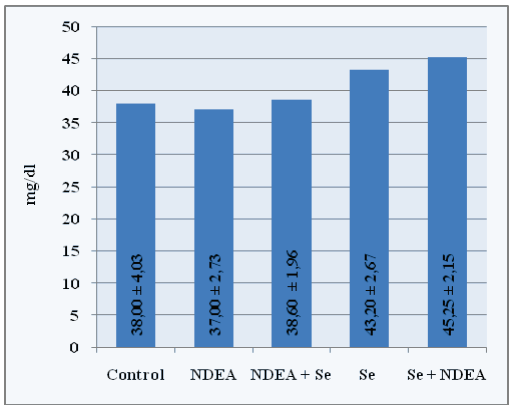

Figure 1. Urea blood levels

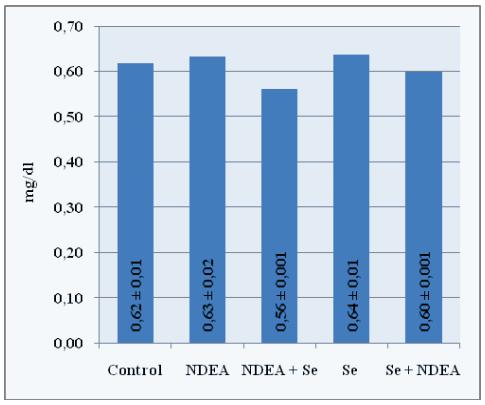

Figure 2. Creatinine blood levels

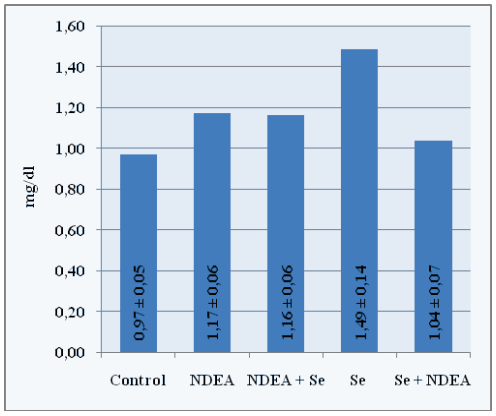

Figure 3. Uric acid blood levels

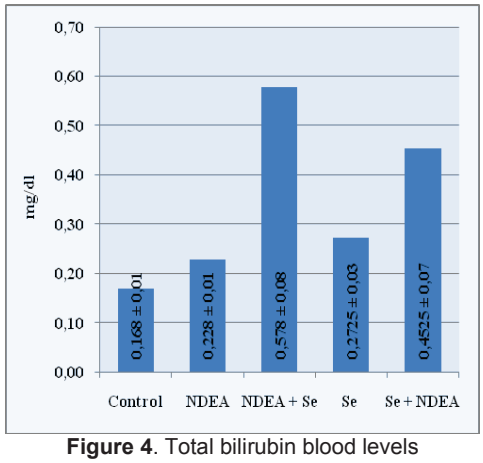

Figure 4. Total bilirubin blood levels

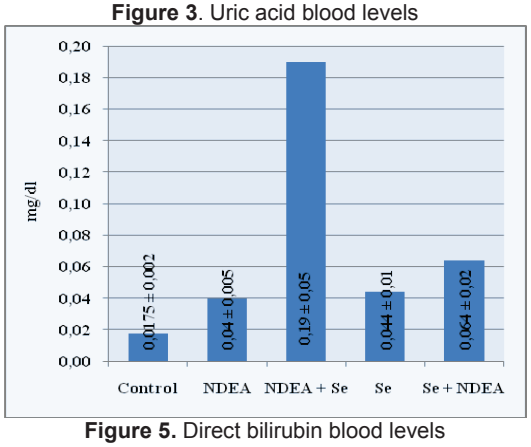

Figure 5. Direct bilirubin blood levels

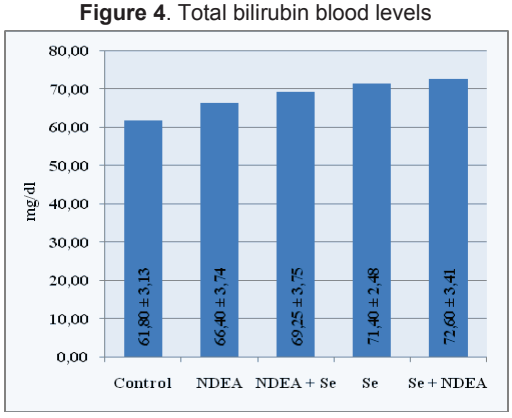

Figure 6. Total cholesterol blood levels

Activities of the liver

NDEA and selenium administration, as single treatments, produced a moderate increase in total and direct bilirubin levels, compared to the control group. The highest total and direct bilirubin levels were found in the NDEA+Se group, fallowed by the Se+ NDEA group, suggesting cholestasis and possible liver damage, if severe enough (Figure 4 and Figure 5).

Total cholesterol blood levels did not differ significantly between groups. The highest level of total cholesterol was noticed in the Se+NDEA group and was not significantly different from the levels found in the NDEA+Se group (Figure 6). HDL-cholesterol and LDL-cholesterol levels showed a different distribution. The highest HDL-cholesterol level was observed in the NDEA+Se group, fallowed by the Se+NDEA group. NDEA and selenium administration, as single treatments, led to a moderate increase in HDL-cholesterol levels, compared to the control group (Figure 7). There were no significant differences between LDL-cholesterol levels corresponding to NDEA, NDEA+Se and Se groups but all these levels were higher than in the control group. The highest LDL-cholesterol level was observed in the Se+NDEA group (Figure 8).

GGT activities did not differ significantly between groups, including control, except for the NDEA+Se group, in which the medium GGT enzymatic activity was over 5-fold higher than in the control group (Figure 9). The high GGT activity observed in the NDEA group, corroborated with the total bilirubin, direct bilirubin, HDL-cholesterol and LDL-cholesterol high values for this group strongly suggests that the animals from this group developed cholestasis and possibly other types of liver disorders, including cirrhosis.

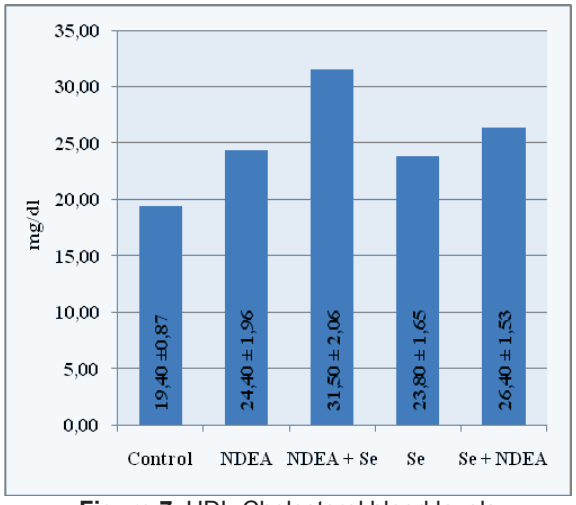

Figure 7. HDL-Cholesterol blood levels

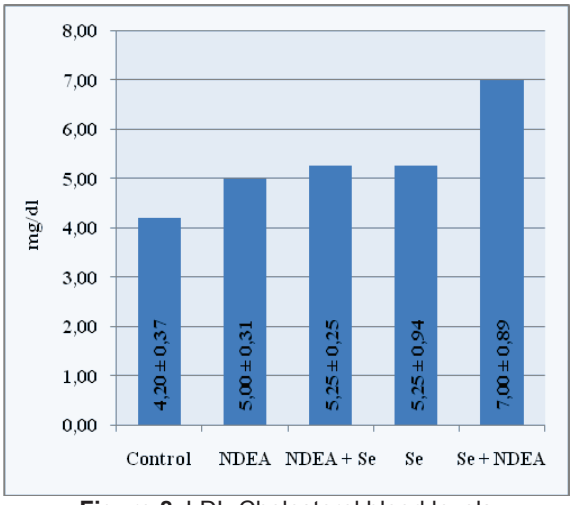

Figure 8. LDL-Cholesterol blood levels

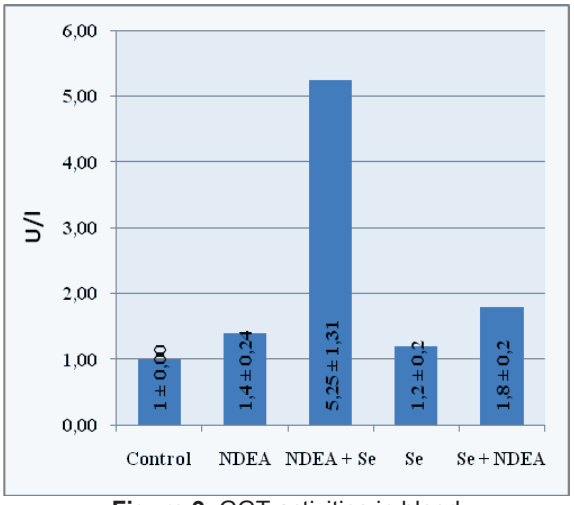

Figure 9. GGT activities in blood

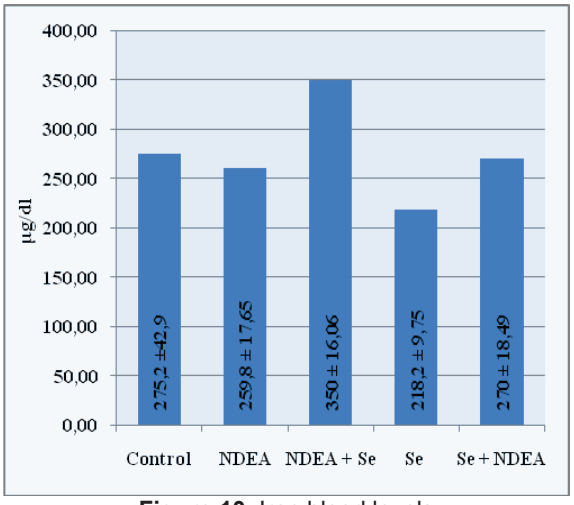

Figure 10. Iron blood levels

Liver cell integrity

There where no significant differences between iron blood levels in NDEA and Se+NDEA groups, compared to the control group, whereas in the case of Se group a moderate decrease was observed, compared to the control group. Iron blood levels were highly increased in the NDEA+Se group, compared to the NDEA and control groups (Figure 10).

The highest ALT and AST activities were observed in the Se group. NDEA produced a moderate increase in ALT and AST activities, compared to the control. ALT activity was higher in the Se+NDEA group, compared to the NDEA+Se group, but AST activity was higher in the Se+NDEA group, compared to the NDEA+Se group.

CONCLUSIONS

At this dose and observational period, NDEA was slightly toxic and induced a significant modification only on some biochemical parameters, compared to the control group (uric acid, total and direct bilirubin, HDL- and LDL-cholesterol, ALT, AST).

Apparently, sodium selenite post-treatment increased NDEA toxicity and was more severe than the pre-treatment.

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