# Effects of nano-selenium on the liver antioxidant enzyme activity and immunoglobolins in male rats exposed to oxidative stress

M. Nasirpour, A.A. Sadeghi*, M. Chamani

Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
*Corresponding author: a.sadeghi@srbiau.ac.ir; Tel: +98 9195579663; Fax: +98 26 44865251

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## Abstract

The aim of this study was to evaluate the effects of nano-particle of selenium on liver antioxidant activity, blood cells count and IgG level in rats exposed to oxidative stress. Twenty four male rats were randomly divided into four treatments with six replicates. Treatments included of: control group gavaged and injected (ip) normal saline; T1: injected (ip) tert butyl hydroperoxide (t-BHP) at 0.2 mmol per kg body weight and gavaged normal saline; T2: gavaged nano selenium (0.3 mg per kg body weight) and injected (ip) normal saline, and T3: injected (ip) t-BHP (0.2 mmol per kg body weight) following gavaged nano-Selenium (0.3 mg per kg body weight). Experimental period was lasted for 35 days and administrations were done each 48 hour. Oxidative stress (T2) increased the liver activities of antioxidant enzymes and also the levels of liver enzymes released in the plasma as compared with other treatments. T3 had lower ($P < 0.05$) enzyme activities than T2 group. T2 had the lowest ($P<0.05$) and T1 had the highest red blood cells, white blood cells, neutrophil and lymphocyte counts. T3 had higher count of red blood cells than T2. Gavage of nano-selenium in stressed rats had no effect on total and differential white cells count compared with stressed rats (T2) or the control group. In stressed rats (T2 and T3), IgG was decreased compared to non-stressed rats. It was concluded that nano-selenium supplementation ameliorate the negative effects of oxidative stress on liver and influence positively in red blood cells count and immunoglobulin production.

**Key words:** Oxidative stress; Nano-selenium; Immunoglobulin; Blood cells; Rat

# Introduction

An imbalance between oxidants and antioxidants causes a condition in the animal body termed as oxidative stress. When oxidative stress occurs, the levels of free radicals increased that can damage cells and impair cells and tissues functions (Al-Gubory *et al.,* 2010). Free radicals adversely influence on antioxidant enzyme activity, immune cells and also Immunoglobolins production (Burton, 2010; Al-Gubory *et al.*, 2010). Antioxidant enzymes activity is necessary to decline the free radicals and inhibition of imbalance between oxidants and antioxidants (Burton & Jauniaux, 2010). These enzymes require selenium as cofactor for activity or building material for stability to reduce the negative effects of free radicals on body structures and functions. During stressful conditions, the need for selenium is higher than normal condition and deficiency may occur (Beckett & Arthur, 2005; Köhrle *et al.*, 2007; Zhang *et al.*, 2008; Chole *et al.*, 2010). Stỳblo *et al.* (2007) showed that selenium deficiency causes a decrease in intracellular glutathione peroxidase activity. In the previous studies (Hoffmann & Berry 2008; Ganabadi *et al.*, 2010; Boostani *et al.*, 2015a,b), the effects of organic and inorganic sources of selenium on immunity, growth, health, and reproduction in human and animals were examined; however, effect of nano-particles of selenium on mentioned parameters remained unknown. Also, the results of these studies are less clear concerning the benefits of Selenium supplementation in animals fed adequate selenium.

Nano-selenium has ubiquitous properties such as high absorbed ability and low toxicity compared to other sources of selenium (Zhang *et al.,* 2008). It was hypothesized that administration of nano Selenium could satisfy the body needs to selenium during oxidative stress and ameliorate the negative effects of stress on antioxidant enzymes activity, metabolism related hormones and immune response. A little information exists concerning the effect of nano-Selenium on mentioned parameters especially in the animals exposed to stress. Therefore, the main purpose of the current study was to evaluate the effect of nano-selenium on the liver antioxidant enzymes activity, blood cells counts, and immunoglobulins levels in male rats exposed to oxidative stress induced by tert-butyl hydroperoxide (t-BHP). Tert-BHP has been used extensively to induce oxidative stress in rat (Yen *et al*., 2004; Hwang *et al*., 2002).

# Materials and methods

Red elemental nano-Selenium was purchased from American Elements Co. (Los Angeles, USA). The average particle size was 42 nm with a purity of 99.95%. Before applying, an aliquot of nano-selenium was poured in the tube containing normal saline with 1% sodium carboxymethyl cellulose (as stabilizer). To disperse the particles, tubes were stirred for 15 min and then put in ultrasonic bath for 25 min. To avoid the aggregation of the particles, fresh suspension was prepared before every use. tert-butyl hydroperoxide (2-Methylpropane-2-peroxol) was provided from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA). Twenty four male Wistar albino rats (120 g body weight) were prepared from the Pasteur Institute (Tehran, Iran). The rats were kept in quarantine for 10 days after entrance and then housed in clean animal room with polycarbonate plastic cages. Animal room had well air-conditioner and controlled temperature (23 °C) and humidity (60%) vehicles and lighting schedule (12 h light/dark). Rats were fed *ad libitum* a laboratory diet suitable for rat and had free access to fresh water. A week was considered for acclimatization to cages environment and thereafter experiment was done. During adaptation period and experiment, rats received free rats selenium pelleted diet. Rats were randomly assigned to four treatments with six replicates. Treatments included of: control group gavaged and injected (ip) normal saline; T1: injected (ip) tert butyl hydroperoxide (t-BHP) at 0.2 mmol per kg body weight (Boostani *et al*., 2015b) and gavaged normal saline; T2: gavaged nano Selenium (0.3 mg per kg body weight) and injected (ip) normal saline, and T3: first gavaged nano-Selenium (0.3 mg per kg body weight) and then injected (ip) t-BHP (0.2 mmol per kg body weight) with one hour elapsed between them. Experimental period was lasted for 35 days and administrations were done each 48 hour.

On day 35 of experiment, rats were fasted overnight with free access to fresh water. Next day, rats were anesthetized with injection (ip) of Ketamine-Xylazine. Blood samples were directly collected into vacuum tubes from heart. A part of blood sample was poured in heparinized-gel containing tubes for separation of plasma. Reminder was poured in the tube containing sodium citrate for measurement of blood red cells and immune cells counts. Then plasma was separated using centrifuge (2500 × *g* for 15 min) and supernatant was collected in the clean tubes and kept at -20 °C. After opening abdominal cavity, liver was removed and placed in clean container and stored at -20 °C.

The glutathione peroxidase (GPx) activity in the liver was assayed based on the procedure of Paglia and Valentine (Paglia & Valentine, 1967). The liver activity of superoxidase dismutase (SOD) was measured based on the procedure described by Beauchamp & Fridovich (1971). The liver activity of catalase was measured using commercial kits (Randox, UK). The levels of alanine aminotransferase (ALP), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the plasma of rats were assayed using commercial kits (Pars Azmoon, Tehran, Iran) and a suitable spectrophotometer (Shimadzu UV-260, Shimadzu Corp, Tokyo, Japan).

The red blood cells (RBC), total and differential white blood cells (WBC) were count using hemacytometer (T-890, Culter, USA). Giemsa-stained blood film was applied to count the differential white blood cells. Plasma IgG was measured using the rat IgG ELISA kit (Life Diagnostics Inc., PA, USA).

To evaluate the normality of collected data, Kolmogorov-Smirnov test was done using SAS Software. Thereafter, analysis of variance was done based on completely randomized design using the GLM procedures of SAS. Tukey test was used for mean comparison at $P < 0.05$.

# Results

The effect of different treatments on the liver activities of superoxide dismutase, glutathione peroxidase and catalase are shown in Table 1. Oxidative stress induced by t-BHP significantly increased the activities of superoxide dismutase, glutathione peroxidase and catalase as compared with other treatments. There was no significant difference ($P > 0.05$) between nSe and the control group for superoxide dismutase activity in the liver, but glutathione peroxidase and catalase activity in the liver of rats received nano-selenium was higher than the control group ($P < 0.05$). Rats in T3 group that received nano-selenium and exposed to stress had lower ($P < 0.05$) superoxide dismutase activity in the liver as compared with T2 group.

**Table 1**. Effects of different treatments on antioxidant enzymes activity in the liver of rats

| ***Treatments*** | Superoxide Dismutase ***IU/mg.pr*** | ***Glutathione peroxidase IU/mg.pr*** | ***Catalase IU/mg.pr*** |
|---|---|---|---|
| Control | 3.36 $^{c}$ | 1.65 $^{d}$ | 4.11 $^{d}$ |
| T1 | 3.53 $^{bc}$ | 2.02 $^{b}$ | 4.79 $^{c}$ |
| T2 | 4.19 $^{a}$ | 2.78 $^{a}$ | 6.73 $^{a}$ |
| T3 | 3.80 $^{b}$ | 1.86 $^{c}$ | 5.52 $^{b}$ |
| SEM* | 0.065 | 0.012 | 0.095 |
| P value | 0.001 | 0.001 | 0.001 |

$^{a, b, c, d}$ *Means in column that possess different superscripts differ significantly (P<0.05).*
*T1: gavaged nano-selenium; T2: exposed to oxidative stress, T3: stressed rats received nano Selenium*
* *SEM: total standard error of means*

**Table 2** Effects of different treatments on liver enzymes activity in the plasma of rats

| ***Treatments*** | ***Alanine aminotransferase IU/mg.pr*** | ***Aspartate aminotransferase IU/mg.pr*** | ***Alkaline phosphatase IU/mg.pr*** |
|---|---|---|---|
| Control | 28.50 $^{c}$ | 35.30$^{c}$ | 46.12 $^{bc}$ |
| T1 | 23.11 $^{c}$ | 36.81 $^{c}$ | 40. 38 $^{c}$ |
| T2 | 44.42 $^{a}$ | 52.64 $^{a}$ | 54.36 $^{a}$ |
| T3 | 34.68 $^{b}$ | 48.50 $^{b}$ | 49.18 $^{b}$ |
| SEM* | 9.269 | 6. 041 | 9.440 |
| P value | 0.001 | 0.001 | 0.001 |

$^{a, b, c, d}$ *Means in column that possess different superscripts differ significantly (P<0.05).*
*T1: gavaged nano-selenium; T2: exposed to oxidative stress, T3: stressed rats received nano Selenium*
* *SEM: total standard error of means*

Table 2 shows the effects of different treatments on liver enzymes activity in the plasma of rats. Rats exposed to oxidative stress had higher plasma activities of Alanine aminotransferase, Aspartate aminotransferase and alkaline phosphatase than other treatments ($P<0.05$). There was no significant difference between rats received nano-selenium as gavage and the control group for these enzymes activities.

**Table 3:** Effects of energy sources and levels on the total and differential of white cells counts

| Item | Red blood cells $\times 10^6$/ml | White cells count $\times 10^3$/ml | Lymphocyte $\times 10^3$/ml | Neutrophil $\times 10^3$/ml | Monocytes $\times 10^3$/ml | Basophil $\times 10^3$/ml |
|---|---|---|---|---|---|---|
| Control | 7.11 [b] | 9.40 [ab] | 6.18 [a] | 2.10 [ab] | 26 [b] | 0.0082 [bc] |
| T1 | 7.75 [a] | 9.69 [a] | 6.23 [a] | 2.21 [a] | 22 [b] | 0.0101 [a] |
| T2 | 6.46 [c] | 8.87 [c] | 5.67 [b] | 1.81 [c] | 31 [a] | 0.0092 [ab] |
| T3 | 7.30 [b] | 9.10 [bc] | 6.06 [a] | 1.96 [bc] | 23 [b] | 0.0072 [c] |
| SEM* | 0.078 | 0.111 | 0.076 | 0.015 | 8.5 | 0.0001 |
| P value | 0.001 | 0.007 | 0.025 | 0.001 | 0.001 | 0.010 |

*a, b, c Means within a column with different superscripts are significantly different (P<0.05).*
*T1: gavaged nano-selenium; T2: exposed to oxidative stress, T3: stressed rats received nano Selenium*
** SEM: total standard error of means*

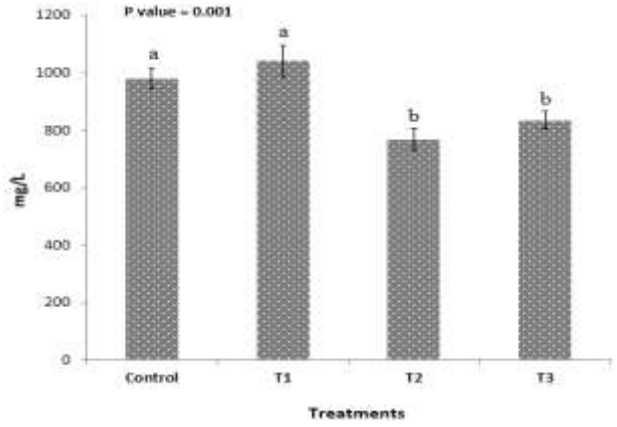


Fig 1. Effects of different treatments on IgG level in the plasma of rats

As shown in Table 3, rats exposed to oxidative stress (T2) had the lowest (P<0.05) and those received nano-selenium (T1) had the highest red blood cells count. There was no difference between T3 and the control group. Administration of nano-selenium in rats exposed to stress increased the count of red blood cells as compared with T2. White blood cells, neutrophil and lymphocyte in group exposed to stress (T2) had the lowest and in group received nano-selenium had the highest count (P<0.05). Gavage of nano-selenium in stressed rats had no effect on these parameters compared with stressed rats (T2) or the control group. There was no significant among the control group, T1 and T3 for monocytes, but difference was appear in rats exposed to oxidative stress.

There were significant differences among treatments for IgG (Figure 1). In stressed rats (T2 and T3), IgG was decreased compared to non-stressed rats. Selenium supplementation had no effect on IgG level in non-stressed and stressed rats.

# Discussion

The main objective of current study was to assess the protective effect of nano-particles of Selenium on the liver and immune system, hence the liver antioxidant activity, liver enzymes appear in the plasma due to damage, immunoglobulins and blood cells were measured. In the literature, there is no report concerning the effects of nano-particles of Selenium on liver antioxidant activity, blood cells count and also immunoglobulins levels in animals exposed to stress.

In this study, rats exposed to oxidative stress show an increase in the activities of SOD, GPx and catalase in the liver. During this condition, antioxidant enzymes such as SOD, GPx and catalase are essential factors for scavenging ROS and reduce the negative effects of them on tissues (Atencio *et al.,* 2009). This finding is in contrast with studies (Zhang *et al.,* 2008; Chole *et al.,* 2010) who reported that human and animals exposed to oxidative stress showed a decrease in the activities of antioxidant enzymes. Discrepancies exist among studies about the effect of oxidative stress on the activities of antioxidant enzymes. The activities of antioxidant enzymes seem to be depend on the duration of exposure, nature and intensity of oxidative stress. Gavage of nano-selenium increased the liver activities of antioxidant enzymes in non-stressed rats, but decreased these activities in stressed rats. Selenium forms selenocysteine, which is the important part of the active site of antioxidant enzyme, glutathione peroxidase and is also necessary for antioxidant enzymes stability and function (Köhrle *et al.,* 2000). A study (Stỳblo *et al.,* 2007) showed that selenium deficiency is associated with significant decrease in GPx activity. In line with our results, Shi *et al.* (2010) and Hao *et al.* (2014) reported that the activities GPx and SOD significantly increased in the tissues of groups fed selenium.

As seen in Table 2, rats exposed to oxidative stress showed an increase in the plasma activities of ALT, AST and ALP. Increase in the level of these enzymes due to oxidative stress has been reported by Ozardali *et al.* (2004), which corresponded with the results of this stud. The plasma activities of these enzymes are the sensitive markers for diagnosis of damage in the hepatic tissue. These enzymes are cytoplasmic enzymes and released into the blood after hepatic cellular damage. During oxidative stress ROS generate and hydroxyl radicals increase peroxidation of fatty acids and finally cell membrane damaged and ruptured. Gavage of nano-selenium had no effect on activities of these enzymes in non-stressed rats, but decreased the activities in stressed rats. In agreement with our results, decrease in the level of these enzymes due to selenium injection has been reported (Ozardali *et al.* (2004).

In the current study, oxidative stress decreased count of red blood cells. In line with this finding, the study of Ghaffari (2008) showed that oxidative stress contributes to the regulation of erythrocyte differentiation and homeostasis. Hematopoietic stem cells and also red blood cells are highly sensitive to high levels of ROS. It was clearly speculated that deficiencies in several scavengers of ROS resulted in anemia (Ghaffari, 2008). In addition, high levels of ROS lead to hemolysis that results in destruction and shorten the life span of red blood cells. Moreover, the process of formation and differentiation of red blood cell is sensitive to ROS (Kong *et al.,* 2004; Lee *et al.,* 2004). Selenium supplementation has been reported to be important in regulating red cell homeostasis and differentiation of erythroid progenitors (Kaushal *et al.,* 2011). As mentioned earlier, during stress, the selenium need increase and deficiency may be occur. Selenium deficiency results in low activity of selenoproteins, which expose cells to oxidative damage. Selenium deficient animals had higher sensitivity to lysis of erythrocyte and methemoglobin formation, which attributed to high levels of ROS.

Total and differential white blood cells affected by oxidative stress, but nano-selenium supplementation had no effect on white cells counts. During stress, the adrenal gland release cortisol (Fantidis, 2010). Cortisol promotes the breakdown protein and inhibits protein synthesis. The immune system depends on the synthesis of immunoglobulins, which is depressed by high level of cortisol and atrophy occur in the lymphoid tissues (Sadeghi et al., 2013). Hence, the production of the leukocytes count decline and immunoglobulins decrease (Post *et al.,* 2003; El-Lethey *et al.,* 2003). It was speculated that selenium supplementation resulted in an enhancement of both cell-mediated and humoral immune responses (Hawkes *et al.,* 2001; Montgomery *et al.,* 2012).

It was concluded that nano-selenium supplementation ameliorate the negative effects of oxidative stress on liver and influence positively in red blood cells count and immunoglobulin production.

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