The protective effect of selenium nanoparticles and selenium against paracetamol

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ABSTRACT
Objective(s): Nanotechnology has enabled researchers to synthesize nanosize particles that possess increased surface areas. Compared to conventional microparticles, it has resulted in increased interactions with biological targets. The objective of this study was to determine the protective ability of selenium nanoparticles compared to selenium against paracetamol hepatotoxicity and GPX concentration.

Materials and Methods: Seventy-two male rats were used in the study, and arbitrarily assigned to six groups. There were 12 rats in each group that six rats were tested in each period. Blood samples were collected from rats for measuring liver enzymes and GPX activity.

Results: The present study shows that paracetamol has a toxic effect on the liver as a result of inducing a marked oxidative damage and release of reactive oxygen species. This was shown by the significant increases in ALP, AST, ALT and LDH activity which was accompanied by significant decrease in GPX activity in the paracetamol-treated group compared to the control, selenium and selenium nanoparticles groups. There was no significant difference between selenium and selenium nanoparticles groups and two period time (15 and 30 day).

Conclusion: Selenium nanoparticles and selenium have protective against paracetamol.

Keywords: GPX activity, Hepatotoxicity, Paracetamol, Selenium nanoparticles

INTRODUCTION
Acetaminophen, the most widely used analgesic in the world, causes severe hepatic necrosis leading to acute liver failure (ALF) after suicidal overdoses [1-3]. Unintentional liver injury from self-medication for pain or fever that leads to daily doses exceeding the 4 g/day package recommendations is also well-recognized [4-7]. Work from Dr. Gillette’s laboratory firmly established the importance of metabolism in acetaminophen toxicity. It was shown that acetaminophen is metabolically activated by cytochrome P450 to form a reactive metabolite that covalently binds to protein; the reactive metabolite was found to be N-acetyl-p-benzoquinone imine (NAPQI), which is formed by a direct two-electron oxidation [8-9].

Selenium was shown to reduce heavy metal toxicities many years ago [10]. The administration of selenium has proved to be highly effective in preventing the toxicity to cadmium [11] and various mercuric compounds [12]. Selenium was shown to protect against the hepatotoxic effect of certain substances causing hepatic necrosis; e.g., carbon tetrachloride [13] and bromobenzene [14]. The protective effects of selenium have been attributed to enhanced activity of glutathione peroxidase, which brings about the destruction of excess lipid peroxides by reducing them to hydroxy acids. In this connection, it is of interest to note that Eaton et al. (1980) [15] and Chung and Maines (1981) [16] have observed increased glutathione levels in livers of rats after single doses of selenite, suggesting the importance of this metabolic effect for detoxification reactions. Studies by Schnell et al. (1983) [17] have shown that selenium pretreatment can also inhibit in a dose-dependent
manner the cytochrome P-450-dependent enzyme system in male rats. Following the administration of selenite (30µmol Se/kg) cytochrome P-450 levels and ethylmorphine N-demethylase activity were significantly decreased in male rats. Additional studies also showed that same pretreatment to prolong the hypnosis of pentobarbital [17].

Recently, the introduction of nanosize elemental selenium produced a highly effective molecular compound, and when compared with SeMet, it showed similar efficacy in increasing antioxidant GPx activity while displaying lower toxicity [18]. Nano-selenium has potent effects on upregulation of GPx, and it yields more efficacious results in the induction of glutathione S-transferase over the short term, but it causes less oxidative stress [19].

These selenium nanoparticles also show high biological activity and good absorptive ability due to the interaction between the nanoparticles and −NH2, C=O, −COO, and −C−N− groups of proteins [20]. Studies on the biological activities of selenium and its nanoforms revealed that hollow spherical nanoparticles of selenium have strong antioxidant properties [21].

Similar studies declared that nano-selenium has the ability to act as an antioxidant with reduced risk of ordinary selenium toxicity [22]. The size of nano-selenium plays an important role in its biological activity, as 5–200 nm nano-Se can directly scavenge free radicals in vitro in a size-dependent fashion [19]. The objectives of this investigation were to examine the ability of selenium and selenium nanoparticles to prevent
the acetaminophen-induced hepatotoxicity and activity of antioxidant enzyme system to explore possible mechanisms by which selenium and selenium nanoparticles can produce its protective action.

MATERIALS AND METHODS

Animals

Young \( (n = 12, \text{age} \ 3 \text{ months}) \) and middle aged \( (n = 12, \text{age} \ 18 \text{ months}) \) Wistar male rats were housed in a thermoneutral environment \( (22 \pm 2 \ ^\circ\text{C}) \), on a 12: 12h photoperiod, and were provided food and water as needed. This investigation was carried out in accordance with the Guide for the Care and Use of Laboratory published by the US National Institute of Health and approved by the institutional animal care committee (NIH publication number 85-23, revised 1996).

Animal treatment

Seventy-two male rats were used in the study, and arbitrarily assigned to 6 groups. Group 1 was the control group, and was given phosphate-buffered saline. Group 2 was the Selenium-treated groups and was given 1mg/Kg Na₂SeO₃ weight intraperitoneally as a single dose for 15 and 30 days. Group 3 was the nano-selenium-treated (Iranian Nanomaterials Pioneers Co.) groups and was given selenium nanoparticles (size 10–40 nm) 0.5 mg/kg body weight intraperitoneally daily for 15 and 30 days. Group 4 was the Selenium-treated groups, which received selenium 1 mg/kg for 15 and 30 days and a single dose of paracetamol 40 mg/Kg on the 5 day. Group 5 was the Selenium-treated groups, which received selenium nanoparticles (size 10–40 nm) for 15 and 30 days and a single dose of paracetamol 40 mg/Kg on the 5 day.
the 5 day. Group 6 was the paracetamol-treated groups and was given paracetamol 40 mg/kg body weight intraperitoneally on 5 day. These groups (2 and 3) were as Controls.

**Assay**

After treatment (15 and 30 day) blood samples were collected from rats for measuring liver enzymes activity (AST, ALT, ALP and LDH and Glutathione peroxidase (GPX) (Pars Azmon co.) As an antioxidant enzyme.

**Statistical analysis**

All the biochemical data were expressed as the mean ± standard error. Differences between the means were tested by independent-sample T-tests for paired data. Statistical analysis was performed by using SPSS, version 21 (IBM, Armonk, NY, USA). A P-value less than 0.05 were considered significant.

**RESULTS AND DISCUSSION**

Measurement of the Alanine aminotransferase (ALT IU/L) activity (Fig 1 ), Aspartate amino transferase (AST IU/L) activity (Fig 2 ), Alkaline phosphatase (ALP IU/L) activity (Fig 3 ) and Lactate dehydrogenase (LDH or LD IU/L) activity (Fig 4 ) in the serum of the 12 tested rat groups revealed that there was a significant decrease in ALT, AST, ALP and LDH activity for paracetamol-treated groups in both two time periods \(P < 0.01\) compared with that of each of group 1 (control), group 3 (nano-selenium-treated group), and group 4 (nano-selenium chromium-treated group) for all liver enzymes activity. As indicated by the results there were less differences between the treated and control groups for all enzymes in comparison with that measured 15 and 30 days injection except paracetamol-treated groups.

According to the results paracetamol enhances the activity of the liver enzymes that represents liver damage. While in control groups (Se and Se nanoparticles) compared to the control group receiving the phosphate-buffered saline liver damage was not observed. Comparison of paracetamol-treated groups can able to reduce the malignant effects of paracetamol.

No significant difference was observed with comparing the protective effects of selenium and selenium nanoparticles, However Selenium element seems to have a more protective effect than selenium nanoparticles.

Se nanoparticles have recently come to be known to have antioxidant effects. These effects are declared to be through increasing activities of Glutathione peroxidase (GPX) as well as causing less oxidative stress. The present study showed decreased activity of GPX following exposure to paracetamol. This was indicated by significant decreases of GPX activity compared to control group. These results are similar to results obtained by other scientists who reported that paracetamol can decreases of GPX and other antioxidant enzymes activity. Paracetamol induced toxicity through increasing cellular oxidative stress and decreasing the activity of antioxidants.
Our results showed that administration of Se nanoparticles and Selenium caused a significant increase in GPX concentration. These findings increase in these enzyme activities suggest a response toward decrease ROS generation. Moreover, it is reported that paracetamol often generates free radicals, which in turn activate O₂⁻ and produce ROS, including hydroxyl radicals, singlet oxygen, superoxide and hydrogen peroxide and consequently lead to DNA damage. Se nanoparticles have recently come to be known to have antioxidant effects. These effects are declared to be through increasing activities of both GPX and glutathione-S-transferase-as well as causing less oxidative stress [23-24].

CONCLUSION
Treatment of rats with nano-selenium (10–20 nm) after paracetamol exposure appeared to counter to the hepatotoxicity status. The administration of selenium and selenium nanoparticles demonstrated beneficial effects upon biochemical alterations, developed in Liver following exposure paracetamol. This proves that selenium and selenium nanoparticles play a significant role in paracetamol hepatotoxicity. It could be concluded that the administration of antioxidants in response to an increased risk of exposure to paracetamol may protect the human body against their harmful effects.

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CONFLICT OF INTEREST
The authors declare that they do not have any conflict of interests.

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