

ORIGINAL ARTICLE

ACUTE TOXICITY OF SODIUM SELENITE IN RODENTS: PATHOMORPHOLOGICAL STUDY

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Summary

The purpose of this study was to investigate the acute toxicity of sodium selenite in mice and rats after *per os* application to establish the relationship between a high toxic dose of sodium selenite and tissue alterations in rats. Increasing doses of sodium selenite (4, 10, 14 and 18 mg/kg) were administered in separate groups of mice and rats. Obtained LD₅₀ values of sodium selenite in mice and rats were in the range of 8.08 to 12.11 mg/kg *po*. In separate groups of rats of both genders, sodium selenite in a dose of 10 mg/kg *po* was applied. Survived animals were sacrificed after the end of day 7 and an increase of fluids in thoracic and abdominal cavities was recorded. Tissue samples of the heart, liver, spleen and kidney were prepared using haematoxylin and eosin (H&E) staining. Histopathological examination revealed the signs of inflammation, haemorrhages, degeneration and rapid loss of normal cell architecture in the heart, liver, spleen and kidney of sodium selenite treated animals. These and other available data suggest the possibility of using environmentally friendly selenite rodenticide. These compounds which possess a different mechanism of action when compared to anticoagulants and an acceptable toxic potential, could improve rodent pest management programs, especially regarding anticoagulant-resistant rodents.

Key words: selenite; Acute toxicity; Mice, Rats; Pathomorphological study.

INTRODUCTION

Chemicals of various types are being used in controlling rodent pests or maintaining their numbers at acceptable level (1, 2, 3). With the development of the first and second generation of anticoagulants, many authors considered that the rodent pests man-

agement programs were significantly improved (4, 5). However, resistance to most of these compounds was reported soon after their introduction, especially in Western Europe and the USA (6 - 11). Despite the above-mentioned disadvantage, bromadiolone and brodifacoum formulations are still widely used in plant and food protection.

Following a current trend of introducing environmentally friendly rodenticides (12), there were attempts of testing the toxicological profile of sodium selenite as a rodenticide. Selenium, a naturally occurring element, frequently in the form of sodium selenite (13), is widely distributed in soil, water, air, vegetation and food (14, 15).

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Selenium is an essential trace element in animals and humans, but in high doses, after acute or chronic exposure, it can cause serious toxic effects (16). Sodium selenate and sodium selenite are used as supplements to poultry and livestock feed to promote growth and prevent selenium deficiency diseases (17). Selenium compounds are generally readily absorbed from the gastrointestinal tract. The absorption does not appear to be homeostatically controlled, since no difference in absorption was observed between selenium deficient and selenium-sufficient rats administered with moderate toxic doses of selenium (18).

Different inorganic and organic forms of selenium may vary greatly in biological activity and toxicity. Therefore, studies involving the investigation of acute and chronic toxicity of selenium have had conflicting results (19).

The aim of this study was to investigate the acute toxicity of sodium selenite in rodents *per os* application and to establish the relationship between a high toxic dose of sodium selenite and pathomorphological changes in some of the vital organs in rats.

MATERIALS AND METHODS

Animals

Adult Swiss mice weighing from 25 to 28 g and Wistar rats weighing from 200 to 250 g of both genders were used in this study. The animals were housed in plastic cages, under standard laboratory conditions (21°C, 12/12-hours light/dark cycle, commercial food and tap water *ad libitum*), before being randomized into groups. Each experimental group consisted of 5 animals. The study protocol was based on the Guidelines for Animal Study No. 282-12/2002 of the Military Medical Academy Ethics Committee, Belgrade, Republic of Serbia.

Chemicals

Commercially available formulation of sodium selenite (99.8%, Alfa Aesar, France) was used. Sodium selenite was dissolved in dimethylsulfoxide (DMSO), immediately before administration.

Acute toxicity

Animals were fasted overnight prior to application of test solutions. Increasing doses of

sodium selenite (4, 10, 14 and 18 mg/kg) were applied in separate groups of mice and rats by *po* route. After the solution had been administered, feed was additionally withheld for 6 hours. The control mice and rats were given DMSO in a dose of 1 ml/kg, *po*.

Lethal outcomes were recorded 24 hours after the administration of sodium selenite. Mean lethal doses (LD₅₀) were calculated according to Litchfield and Wilcoxon test (20).

General toxicity and histopathological examination

In two separate groups of rats of both genders, sodium selenite in a dose of 10 mg/kg *po* was applied. Survived animals were sacrificed after the end of day 7. The heart, liver, spleen and kidney were excised and their samples were fixed in 10% neutral formalin for 5 days. Tissue samples were dehydrated in graded alcohols, xylol and embedded in paraffin blocks. Finally, 2-µm thick paraffin sections were stained by haematoxylin and eosin (H&E) and analyzed using Olympus-2 microscope (Tokyo, Japan).

RESULTS

Evaluation of lethal outcomes and general toxicity

The results of sodium selenite acute toxicity examination are shown in Table 1. There are only moderate differences in acute toxicity of sodium selenite concerning both species and genders. In both species, sodium selenite exerted more prominent lethal effect in males.

Table 1. 24hrs LD₅₀ values of sodium selenite in mice and rats

Species	Gender	LD ₅₀ (mg/kg <i>po</i>) ¹	95% confidence limits
Mice	Male	8.08	4.54 - 14.36
	Female	11.51	5.85 - 22.65
Rats	Male	10.29	4.64 - 22.80
	Female	12.11	5.47 - 26.82

¹Calculated by the Litchfield and Wilcoxon test.

During first 24 hours after intoxication (acute toxicity study), gastrointestinal disturbance, hypersalivation, muscle spasm, decreased food and water consumption and lethargy were seen in animals treated with all the doses except the lowest one.

In the survived animals, from the 2nd to the 7th day period of observation, no significant changes of the general health could be seen. General motor activity, coordination as well as food and water consumption were preserved in the most of survived animals.

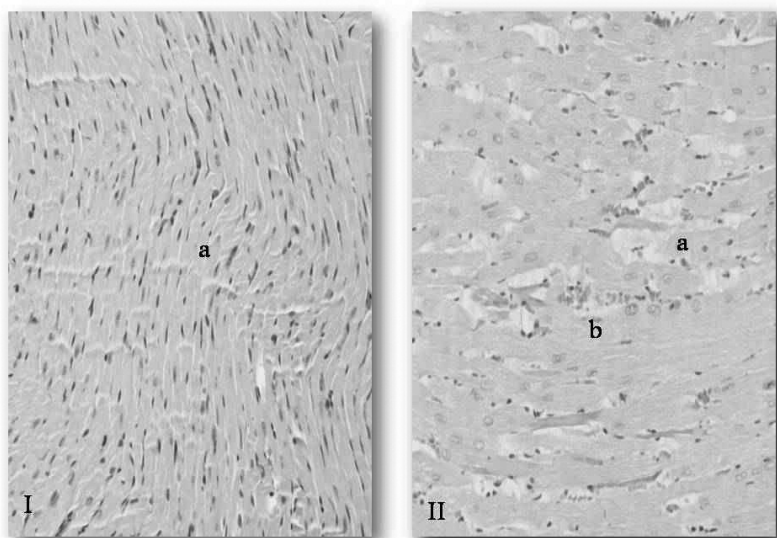
Moreover, their hair, skin, visible mucosa and muscle tonicity were without any visible changes.

An increase of fluids in thoracic and abdominal cavities was recorded after sacrifice. Various organs (heart, liver, lungs, spleen, gut, kidneys, urinary bladder and adrenal glands) had normal morphological appearance.

Evaluation of microscopic findings

Single administration of sodium selenite caused severe, diffuse and massive degenerative and vascular alterations in rats of both genders. Affected cardiomyocytes displayed extensive sarcoplasmic vacuolisation or pale eosinophilic sarcoplasm and lack cross-striations. In these irregular, round to ovoid cells, nuclear polymorphism (large, round to rectangular shapes and prominent nucleoli) was present. The affected areas were observed in the subepicardium, myocardium and endocardium. Thickening of the blood vessels as well as vacuolisation of endothelial cells was observed, too. The interstitial haemorrhages appeared uniformly in each of the examined sections. Haemorrhages were located in the middle myocardial and subendocardial areas (see Figure 1).

Figure 1. Histopathological changes in the heart of rats acutely poisoned by sodium selenite



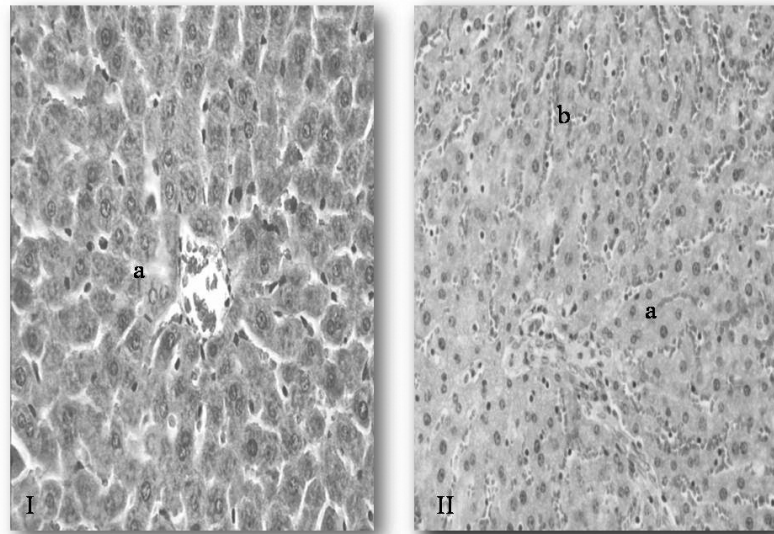
(I) Control group: a) Normal histological structure of the cardiac tissue.

(II) Sodium selenite treated group: a) Extensive vacuolar degeneration of the cardiomyocytes, b) Focal interstitial haemorrhages. Haematoxylin and eosin staining, magnification 200x.

Hepatic injuries ranged from vacuolar degeneration to focal necrosis of small groups of hepatocyte and were associated with focal, moderate haemorrhages. These alterations were most prominent in the centrilobular areas with the

damaged hepatocytes completely encircling the central vein. The moderate oedema, hyperaemia and haemorrhagic foci with discrete accumulation of inflammatory cells were present in the sinusoids and in the perisinusoidal spaces (see Figure 2).

Figure 2. Histopathological changes in the liver of rats acutely poisoned by sodium selenite



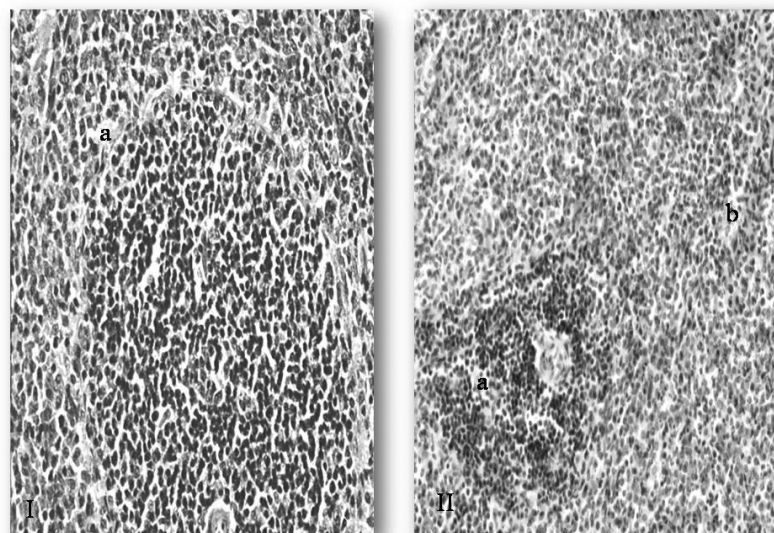
(I) Control group: **a)** Normal histological structure of the hepatocytes.

(II) Sodium selenite treated group: **a)** Vacuolar degeneration of the hepatocytes, **b)** The moderate oedema, hyperaemia, haemorrhages and focal accumulation of inflammatory cells. Haematoxylin and eosin staining, magnification 200x.

A severe and rapid depletion of lymphocytes was present in the spleen, with higher lymphocytes density in the outer than in the inner parts of the lymphatic nodules. These affected areas contained an increased number of the plasma cells when compared

to control. Vacuolisation and a higher incidence of picnotic nuclei in the endothelial cells of the central arterioles could be seen. Vascular alterations, like focal and massive haemorrhages, were found in the red pulp (*see Figure 3*).

Figure 3. Histopathological changes in the spleen of rats acutely poisoned by sodium selenite



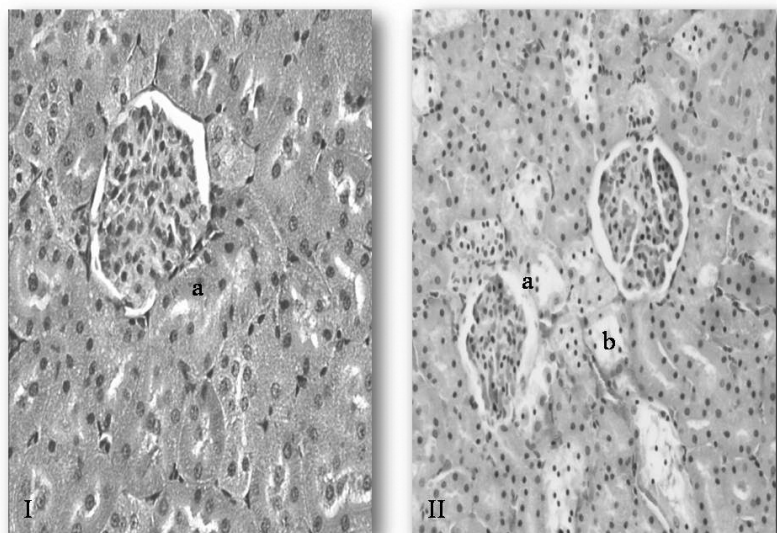
(I) Control group: **a)** Normal histological structure of the lymph node.

(II) Sodium selenite treated group: **a)** Severe depletion of lymphocytes, **b)** Focal haemorrhages in the red pulp. Haematoxylin and eosin staining, magnification 200x.

Development of membranous glomerulonephritis in the kidney tissue was detected. Degeneration and focal necrosis of proximal tubular cells were seen as well. The presence of interstitial oedema,

hyperaemia, haemorrhages and accumulation of inflammatory cells (neutrophils, lymphocytes and plasma cells) was recorded focally in all examined sections (see Figure 4).

Figure 4. Histopathological changes in the kidney of rats acutely poisoned by sodium selenite



(I) Control group: a) Normal histological structure of the renal tissue

(II) Sodium selenite treated group: a) The membranous glomerulonephritis b) Degeneration of the proximal tubular cells, interstitial oedema, hyperaemia, haemorrhages and accumulation of inflammatory cells. Haematoxylin and eosin staining, magnification 200x.

DISCUSSION

Signs of acute selenium toxicity in humans are garlic or sour breath odour, gastrointestinal disturbance, restlessness, hypersalivation, muscle spasms, haemolysis, liver necrosis, cerebral and pulmonary oedema, coma and death (19, 21- 23).

Similarly, animals given lethal doses acquire a garlicky breath, show signs of restlessness and fear, followed by somnolence. Clinical signs are characterized by abnormal behavior, respiratory difficulty, gastrointestinal upset and sudden death. Abnormal posture and depression, anorexia, unsteady gait, diarrhoea, colic, increased pulse and respiratory rates, frothy nasal discharge, moist rales and cyanosis may be noticed. Death usually follows within a few hours of consumption or injection. The major lesions are lung oedema and congestion, and necrosis of multiple organs, including lungs, liver and kidneys (24).

Results of some previous studies show that the

acute toxicity of selenium compounds was directly proportional to their aqueous solubility (25). In animals, the most toxic orally taken selenium compounds appear to be sodium selenite and sodium selenate (26). The highly soluble sodium selenite was 900 times more toxic than the insoluble elemental selenium.

Many selenium compounds are very toxic and induce lethal effect in laboratory animals in single doses as small as 1.5 - 6 mg Se/kg body weight, concerning elemental selenium. It was shown that selenite killed most of the intraperitoneally treated rats within 2 days at the dose range of 3.25 - 3.5 mg Se/kg. The corresponding lethal doses for selenate and for selenocysteine were 5.5 - 5.75 mg Se/kg and 4 mg Se/kg, respectively (27). However, some other compounds, such as selenium sulfide and dimethylselenide, are less toxic.

In our experiments, sodium selenite administered in a dose range of 4 to 18 mg/kg *po*, increased mortality rate in a dose dependent

manner. The obtained LD₅₀ values in mice and rats are comparable to those from literature (27).

The no-observed-adverse-effect level (NOAEL) of sodium selenite was estimated 0.4 mg of Se/kg body weight in rats and 0.9 mg Se/kg body weight in mice (17). Thus regarding these and the acute toxicity data from our study, 10 - 20 fold increase of the dose is needed for lethal outcome.

Selenium is distributed throughout the body, but the highest amounts are accumulated in the liver, kidneys, and muscles (28).

It is well known that selenium is an integral part of the enzyme system glutathione peroxidase, which protects intracellular structure, especially DNA, against oxidative damage (23, 29). We noticed prominent histopathological alterations in the heart, liver, spleen and kidney. Our histopathological findings showed the signs of inflammation, haemorrhages, degeneration and rapid loss of normal cell architecture in all the examined tissues of sodium selenite treated animals.

Mice which received repeated high oral dose of selenocystine (20 mg/kg) for 10 days showed a significant rise of aspartate aminotransferase and alanine aminotransferase, as well as increased prothrombin time (30). Most of our macroscopic and histopathological findings could be explained by the above-mentioned facts.

Biochemical aspects of sodium selenite toxicity have been studied in different *in vitro* systems. In general, selenite concentrations of approximately 20 - 100 µmol/L (1.6 - 7.9 mg of Se/L) damage or lyse most cell types. It was demonstrated that redox changes are of major importance for the cellular lysis in the isolated hepatocytes. The severe redox changes can be explained by redox cycling of autooxidizable selenium metabolites (31, 32). Other toxic mechanisms are associated with depletion of reduced glutathione, protein synthesis inhibition (33), depletion of S-adenosylmethionine (34), and a general replacement of sulfur by selenium in cellular metabolism (35).

Higher concentrations of selenium in soil and water near the power plants (coal mines, thermal plants), as well as large farms (where it is used as supplements to poultry and livestock feed) are registered. Thus, selenium becomes a pollutant which can potentially present a health hazard. However, as far as rodenticides are concerned, strict legislation concerning their utilisation makes this subject of no special concern.

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