



Alteration of Energy Metabolism in Sodium Selenite Induced Rat Model

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Abstract

Selenium is a metalloid present as selenide minerals, selenite and selenite salts. Selenium has many good benefits as it act as antioxidant, protects DNA damage, supports immune system etc., besides them if the selenium limit exceeds it causes toxic effects. In this contest we investigated the acute toxicity of sodium selenite in rat model by inducing sodium selenite. We had obtained average value of Lethal Dose (LD₅₀) as 7.14mg/kg. Rats were divided into four groups consisted of ten rats in each group. Group (1) as control rats treated with saline, Group (2) as single dose induced Sodium selenite Group (3) as double dose induced Sodium selenite, Group (4) as multiple dose induced Sodium selenite. Animals were sacrificed and different regions such as Brain, Liver, Kidney and Testis were immediately isolated, frozen in liquid nitrogen and were stored at -40°C until analyses. The selected enzymes such as Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH), Succinate dehydrogenase (SDH), Glutamate dehydrogenase (GDH), Adenosine triphosphatases (ATPases), Aspartate aminotransferase (AAT) and Alanine amino transferases (ALAT) were examined in the experimental animals. The present study revealed the toxic effect of selenium in different doses.

Keywords

Sodium selenite, acute toxicity, LD₅₀, dose dependent.

INTRODUCTION:

Selenium is an essential trace element occurring solid substance which is distributed in earth crust, which has both properties as essential and toxic. Both its deficiency and excess are harmful to humans, which leaves its optimum utility. Selenium also plays an important role in redox reactions. The primary inorganic form of selenium is sodium selenite, which is found in natural water and soil. Sodium selenite is inactive and requires organification to exert its biological actions. The selenium containing enzyme glutathione peroxidase is present in most body tissues and catalyses the

breakdown of the highly reactive metabolite hydrogen peroxide (Johnson, 1992). As a micronutrient, Se it plays its multiple biological functions role in various regulation of vitamins absorption, participation in electron transfer, body metabolism regulation, affection towards the reproductive function of humans and animals, antitumor, free radical scavenger, antiaging, and antagonism of toxic elements, playing important roles in improvement of the human body's immune system and the prevention of various diseases. (Brown and Arthur, 2001; Kohrle et al., 2000; Li et al., 2009; Zhao et al., 2009). Certain trace elements

demonstrate the properties of nutritional and toxicological perspectives and consequently give rise to questions about the toxicity of essential dietary minerals. Selenium is the one typical example of this toxicity-essentiality paradox.

It is obtained from food, and the recommended dietary allowance is 55 µg/d for persons 14 years or older, with a tolerable upper intake limit of 400 µg/d. (ATSDR, 2003; IOM, 2000). The amount of selenium available in a diverse diet with meat, grains, vegetables, and nuts is typically sufficient to negate the necessity for supplementation. (Levander, 1991) Selenium toxicity can occur with acute or chronic ingestion of excess selenium. Symptoms of selenium toxicity include nausea; vomiting; nail discoloration, brittleness, and loss; hair loss; fatigue; irritability; and foul breath odor (often described as “garlic breath”) (Fan and Kizer, 1990; Nuttall, 2006; Yang et al., 1983). Extremely high intakes of selenium can cause severe problems, including difficulty breathing, tremors, kidney failure, heart attacks, and heart failure (NIH, 2019). Some researchers showed that sodium selenite induced severe oxidative damage, especially in the brain regions of rats (Chen and Zhou, 2019), selenite-induced cataract rat models (Zhu et al 2011). Selenocompounds, especially sodium selenite, can display pro-oxidant properties that may be toxic (Spallholz, 1997; Stewart et al., 1999). Indeed, selenium also known as toxic element in animal poisoning by selenium-rich plants (Barceloux, 1999). To emphasise the core issue of selenium toxicity, it was felt necessary to carry out a comprehensive investigation about the selenium toxicity in a mammalian rodent model. The present research has been taken up to elucidate the toxic effect of Sodium selenite studying energy metabolisms in liver, brain, kidney and testis under sodium Selenite exposure rat model.

MATERIAL AND METHODS:

Experimental animals

Adult male Wistar rats (150±25g) were used for the present study. The experimental rats were housed in polypropylene cages under laboratory conditions of 28±20°C temperature, with relative humidity of 75%, and 12 h light/dark cycle. The rats were given standard pellet diet and water ad libitum. The rats were maintained according to the ethical guidelines for animal protection and welfare with the CPCSEA in its resolution No: 9/IAEC/SVU. These rats were fasted overnight before they were subjected to sodium selenite treatment.

Lethality (LD50)

Toxicity evaluation of sodium selenite was done by “probit method” of Finney (1971). Rats were treated

with different –doses of Sodium Selenite by oral intubation dose and mortality were noted. LD50 was the dose at which 50% off test animals were killed. After determination of LD50 dose, 1/5 of the LD50 was selected as Sublethal concentration for subacute Studies.

Experimental protocol

Healthy adult animals were divided randomly into four groups, with ten animals each. Sodium selenite was dissolved in distilled water and administered in the required doses through a polyethylene stomach tube connected to a metal needle with blunt tip. Isovolumetric distilled water was administered to the control animals. The first group of animals were used as controls. To the animals of second group, Single dose of sodium selenite was administered orally on first day. Double dose was given to third group of animals on 1st and 3rd day. Similarly multiple doses (i.e., on 1st, 3rd, 5th and 7th days) were given to the fourth group of animals.

Isolation of tissues

After stipulated duration, the animals were sacrificed by cervical dislocation and different brain regions such as Brain, Liver, Kidney and Testis were immediately isolated, frozen in liquid nitrogen and were stored at -40°C until analyses.

Biochemical Analysis

The selected enzymes such as Lactate dehydrogenase (LDH) was measured by Srikanthan and Krishnamoorthy (1955), Malate dehydrogenase (MDH) and Succinate dehydrogenase (SDH) were determined by Nachlas et al. (1960), as modified by Prameelamma and Swami (1975), Glutamate dehydrogenase (GDH) activity levels by method of Lee and Lardy (1965), Adenosine triphosphatases (ATPases) was estimated by Desai and Ho (1979). Aspartate aminotransferase (AAT) and Alanine amino transferases (ALAT) were both assayed by Reitman and Frankel (1957).

Statistical analyses

All the data presented are mean, Standard error and Analyses of Variance was done using SPSS statistical software

RESULT:

The LD₅₀ value was determined as 7.14 mg/kg body weight and 1/5 of LD₅₀ value is selected as sub lethal concentration of 1.43 mg/kg body weight in induced rat model. When compared to the controls, Se induced acute toxicity caused significant elevation in LDH, GDH AND AAT in all the tissue regions and the highest increase in some enzyme activity was noted in multiple dose induced Se-toxicity. Whereas SDH, ALAT, MDH and ATPases activities were decreased in

all the selected tissue regions in Se -induced animals compared to Controls.

Table.1: Alterations in the content of LDH, SDH, MDH, GDH, ALAT, AAT, ATPases in different selected tissues of Sodium selenite-induced rat model

Enzyme activity	Tissue selected	Control	Single Dose	Double Dose	Multiple dose
LDH (μ moles of formazan formed / mg protein /hour).	Brain	1.820 $\pm$ 0.336	2.110 $\pm$ 0.213	2.341 $\pm$ 0.251	2.573 $\pm$ 0.252*
	Liver	5.642 $\pm$ 0.491	6.029 $\pm$ 0.703	6.460 $\pm$ 0.522*	7.246 $\pm$ 0.614*
	Kidney	2.340 $\pm$ 0.335	2.749 $\pm$ 0.293	2.874 $\pm$ 0.211*	3.176 $\pm$ 0.282*
	Testis	0.168 $\pm$ 0.016	0.265 $\pm$ 0.022*	0.302 $\pm$ 0.026*	0.409 $\pm$ 0.045*
SDH (μ moles of formazan formed / mg protein/hour).	Brain	0.926 $\pm$ 0.073	0.727 $\pm$ 0.070*	0.320 $\pm$ 0.032*	0.483 $\pm$ 0.048*
	Liver	0.340 $\pm$ 0.030	0.259 $\pm$ 0.022	0.094 $\pm$ 0.012*	0.078 $\pm$ 0.008*
	Kidney	0.757 $\pm$ 0.059	0.554 $\pm$ 0.048	0.359 $\pm$ 0.030	0.231 $\pm$ 0.024*
	Testis	0.686 $\pm$ 0.052	0.566 $\pm$ 0.049	0.446 $\pm$ 0.036	0.425 $\pm$ 0.043
MDH (μ moles of formazan formed / mg protein/hour).	Brain	0.672 $\pm$ 0.045	0.561 $\pm$ 0.043	0.388 $\pm$ 0.028	0.253 $\pm$ 0.023*
	Liver	0.717 $\pm$ 0.062	0.634 $\pm$ 0.049	0.564 $\pm$ 0.045	0.397 $\pm$ 0.032*
	Kidney	0.670 $\pm$ 0.045	0.610 $\pm$ 0.052	0.548 $\pm$ 0.040	0.486 $\pm$ 0.040*
	Testis	0.593 $\pm$ 0.039	0.472 $\pm$ 0.038	0.365 $\pm$ 0.016	0.252 $\pm$ 0.030*
GDH (μ moles of formazan formed /mg protein /hour).	Brain	0.283 $\pm$ 0.041	0.324 $\pm$ 0.021	0.360 $\pm$ 0.033	0.437 $\pm$ 0.043
	Liver	0.246 $\pm$ 0.018	0.290 $\pm$ 0.026	0.304 $\pm$ 0.036	0.363 $\pm$ 0.032
	Kidney	0.217 $\pm$ 0.022	0.255 $\pm$ 0.028	0.281 $\pm$ 0.029	0.312 $\pm$ 0.031
	Testis	0.185 $\pm$ 0.017	0.22 $\pm$ 0.019	0.253 $\pm$ 0.022	0.273 $\pm$ 0.023*
ALAT (μ moles of pyruvate formed / mg protein/hour).	Brain	4.500 $\pm$ 0.472	5.754 $\pm$ 0.615	6.146 $\pm$ 0.542	6.551 $\pm$ 0.629*
	Liver	5.832 $\pm$ 0.577	7.752 $\pm$ 0.750	8.841 $\pm$ 0.929*	9.540 $\pm$ 0.758*
	Kidney	3.463 $\pm$ 0.389	4.32 $\pm$ 0.437	5.016 $\pm$ 0.539	5.773 $\pm$ 0.561*
	Testis	3.691 $\pm$ 0.370	4.308 $\pm$ 0.428	5.98 $\pm$ 0.604	6.513 $\pm$ 0.486*
AAT (μ moles of pyruvate formed / mg protein/hour).	Brain	3.811 $\pm$ 0.367	4.708 $\pm$ 0.476	5.864 $\pm$ 0.671*	6.518 $\pm$ 0.800*
	Liver	4.428 $\pm$ 0.405	5.550 $\pm$ 0.582	6.523 $\pm$ 0.723*	6.964 $\pm$ 0.528*
	Kidney	2.284 $\pm$ 0.256	2.847 $\pm$ 0.342	3.498 $\pm$ 0.314	3.948 $\pm$ 0.403*
	Testis	3.256 $\pm$ 0.300	3.744 $\pm$ 0.425	3.958 $\pm$ 0.428	4.428 $\pm$ 0.427*
ATPases (μ moles of inorganic phosphate formed / mg protein /hour).	Brain	8.719 $\pm$ 0.876	7.703 $\pm$ 0.767	7.323 $\pm$ 0.749	7.109 $\pm$ 0.763
	Liver	4.015 $\pm$ 0.439	3.397 $\pm$ 0.338	2.605 $\pm$ 0.287*	2.167 $\pm$ 0.194*
	Kidney	8.445 $\pm$ 0.855	7.122 $\pm$ 0.611	6.452 $\pm$ 0.617*	5.211 $\pm$ 0.381*
	Testis	3.539 $\pm$ 0.391	3.062 $\pm$ 0.268	2.718 $\pm$ 0.310*	2.265 $\pm$ 0.217*

All the values are mean, $\pm$ SE (Standard Error) of ten individual observations.

Values in '()' parenthesis are % change over saline control

* Values are significant at $P < 0.05$ in Scheffe test.

DISCUSSION:

Energy metabolism occupies a key position in the metabolic machinery and its modulation leads to variations in the energy budget of the cell. Glucose molecule undergoes a series of enzyme catalyzed reactions to release energy rich electrons which are captured by coenzyme and pass to the cytochrome system to convert chemical energy through phosphorylation from ADP to ATP. Activities of selected dehydrogenases namely LDH, SDH, MDH and GDH were determined in brain, liver, kidney and testis of control and sodium selenite treated rats. A significant increase in LDH except in testis activities and decrease in SDH activities were observed in multiple dose of all the selected tissues of rat when compared to controls in response to sodium selenite administration. LDH catalyzes the reversible

oxidation of lactate to pyruvate in the terminal step of glycolysis. It is located at a strategic point between glycolysis and TCA cycle and occurs in five different isozymic forms. It forms as a center of delicately balanced equilibrium between anabolism and catabolism of carbohydrates. It is also involved in gluconeogenesis in tissues in which lactate is converted to glycogen through glycogenesis.

Elevation in the specific activity of LDH was observed in the present investigation and this may be due to diminished TCA cycle enzymes activities. Similar increase in LDH activity was reported in isolated rat hepatocytes with sodium selenite (Park and Whanger, 1995) and in HepG2 cells (Shen et al., 1999). The LDH activity increases during conditions favouring anaerobic respiration to meet energy demands, when aerobic respiration is lowered

(Murray et al., 1995). Selenium inhibited the activities of oxidative enzymes like SDH and MDH (Table 1) with a subsequent fall in energy production indicating the prevalence of anaerobiosis. Augmented anaerobiosis has been found to be associated with elevated LDH activity in animal tissue. Thus there appears a shift in the carbohydrate metabolism from aerobic to anaerobic condition during selenite stress. Selenium causes severe necrosis and induction of haemorrhages in different tissues, might have contributed to the augmentation of LDH activity. Diminished cellular oxidations leading to anaerobiosis could be one of the reasons for the elevation of LDH in the tissues investigated (Table 1). From the results it can be visualized that there is a rapid depletion of SDH activity denotes fluctuations of oxidative metabolism and also reflects the turnover of carbohydrates and energy output. Low operation of glycolytic pathway and reduction in Pyruvate feeding into TCA cycle corroborates with the reduced activity levels of SDH. Significant reduction in the dehydrogenase activity might also be due to the direct interaction of selenium with mitochondria. It is well documented that selenium will react with thiol groups of inner mitochondrial membranes and biochemical lesions in liver mitochondria and exerts inhibitory effects on various dehydrogenases. On par with SDH activity MDH activity was also inhibited in the selected tissues of rat during sodium selenite stress (Table 1). The drop in MDH activity denotes fluctuations of oxidative metabolism and also reflects the turnover of carbohydrates and energy output. Increased GDH activity was observed in the experimental rats treated with sublethal dose of sodium selenite, when compared to the control rats (Table 1). Elevation in GDH activity in stressed rats indicates increased oxidation of glutamate. AAT and ALAT are the two most studied aminotransferases in animal tissues catalysing the exchange of amino groups between glutamate and aspartate and between glutamate and alanine. AAT catalyses the interconversion of aspartic acid and α -ketoglutarate and pyruvate /glutamate. In our study both transaminase activities were determined in control and experimental rats. A significant increase in the activity levels of these enzymes was observed in double and multiple doses of all tissues of rats when compared to controls in response to sodium selenite treatment, this was supported by another research (Hasegawa, et al., 1996). The enhanced aminotransferase activity in the present study under selenium intoxication indicates effective transamination by these enzymes. Elevated activities of aminotransferases during stress would lead to increase feeding of ketoacids into TCA cycle

thereby affecting oxidative metabolism. A high concentration of selenium can damage the structure and function of mitochondria, and thus influence their metabolism (Chen et al., 1997). ATPases are integral enzyme systems of mitochondria, hence any change damage caused to mitochondrial structure may alter the ATPase activity thereby impairing energy metabolism. Total ATPase activity was determined in our present investigation. A significant decrease in the activity levels of the enzyme was observed in double and multiple doses of all the tissues of rat in response to sodium selenite treatment. Since the elevation of phosphatases is considered as a marker of disease or pathological condition, it is likely that selenium under sub-acute exposure should result in some degree of tissue damage, even at sublethal concentration.

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