

## Effect of Sodium Selenite on Kidney Function Test of Rabbits

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### Abstract

This study was conducted to evaluate the effect of Sodium Selenite at different dosage levels mixed with water in rabbit. Fifteen rabbits (120 day old) were purchased from Tolinton market, Lahore. They were divided into three groups having three replicas (5 rabbits each). One group was kept as un-medicated control A. While the other Groups such as B and C were medicated with sodium selenite in a single dose at the rate of 2 and 3 mg/kg of body mass mixed in water for 20 days consecutively. Control diet Group "A" received basal diet without any supplementation. Blood samples were collected on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day of dietary treatment from each group. Various serological parameters of kidney functioning enzymes (RFT) such as Creatinine and Urea were determined by using Semi automated Chemistry Analyzer. A significant increase in Creatinine and Urea levels ( $p < 0.05$ ) in rabbits of various treated groups was recorded after applying statistical ANOVA test. The results revealed that Sodium selenite alters the serum creatinine and urea levels by affecting the kidney and cause renal toxicity in rabbits.

**Keywords:** Rabbits, Serological parameters, Kidney function enzymes test, Creatinine test, Urea test.

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## INTRODUCTION

Essential minerals have a metabolic role in the body and are divided into two groups such as the major elements (cadmium, phosphorous, potassium, Sodium, chlorine and magnesium) and trace minerals (iron, iodine, copper, manganese, selenium and zinc) (Donald *et al.*, 1987). Selenium is a trace element and act as cofactor in enzymatic functions and other metabolic processes (Rooney, 2007) and plays a key role in functions of immune system, regulation of thyroid hormone metabolism and enzymatic antioxidant defenses (Iglesias *et al.*, 2013). Selenium is an important nutrient in the nutrition of organisms. It is nonmetallic element that causes toxicity in different types of animals when it is ingested by animals from various sources such as plants growing in soils (Franchini and Bertuzzi, 1991).

Selenium is washed out from the soil by the water; due to this reason the plants are able to take selenium form water along with the soil. The concentrations of selenium in plants vary; those plants are able to accumulate selenium against a concentration gradient are known as selenium

accumulators, which is responsible for the transport of selenium to the people at the end of food chain (Dumont *et al.*, 2006). Selenium passes placental barriers as well as through mother milk to suckling due to transportation in the organism to various tissues and cells, after ingestion it is reached to the soil through feces (Allen and Miller, 1981). Soil bacteria are responsible for conversion of selenium to selenite. Interest is increasing in the selenium compound due to their biological and toxicological importance and in particular, because of their anticancer activities (Combs, 2001).

The high concentration of selenium causes toxic effects in organisms. Chronic selenium poisoning, introduce selenosis in humans and produces alteration in the liver in China (Gailer *et al.*, 2000). Sensitivity to viral infections is increased by the Selenium insufficiency and antioxidant status. Kashin-Beck disease (KBD) is the syndrome caused by lack of selenium together with the iodine deficiency and causes severe disorders such as atrophy, degeneration and necrosis of cartilage tissues (Hol *et al.*, 2001). The oxidative stress resulted from selenium

deficiency results in the development of abnormal oxidation reduction system in organs and cells (Bordoni *et al.*, 2007).

Toxic levels of Se (10-20 ppm) are more than 100-fold higher the nutritional requirements. Usually Se doses lower than 3-5 ppm in animal feed are not associated with toxicity (Surai *et al.*, 2002a). The consumption of higher levels of Se can cause selenosis, characterized by hair loss, gastrointestinal upsets, white blotchy nails, fatigue, irritability, and mild nerve damage in both humans and animals. Sodium selenite is toxic than less soluble elemental selenium, selenium sulfide and selenium disulfide (Koller *et al.*, 1986).

The purpose of this study was to notice the toxic effect of sodium selenite on kidney function test in rabbit.

## MATERIALS AND METHODS

### Experimental Animals

The experiment was conducted on 120 day old (two months) clinically healthy fifteen rabbits with a mean weight of 1kg. These rabbits were purchased from the Tolinton market, Lahore, Pakistan. The rabbits were divided into three groups. Each group contains 5 animals. One of these groups (A) was kept as a control group. Other two (B&C) were served as experimental groups. Rabbits of group B and Group C were identified from each other by ribbon tags of orange and green color respectively.

### Acclimatization

The rabbits were allowed to acclimatize to laboratory condition in well-ventilated cage for 5 days. They had access to feed and water all the time. They were kept under standard conditions of temperature (37 °C) and humidity (40-50%). Care of the animals was taken as per guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC) and the study was approved by Institutional Animal Ethics Committee.

### Toxicant

Selenite was used in the form of Sodium selenite. This toxicant was mixed with water in varying amount. Sodium selenite was purchased from BDH chemicals Ltd.

### Dietary plan

Control group (A) was fed with simple diet mixed with water. Experimental diets mixed with water containing 2mg/kg and 3mg/kg of Sodium selenite were given to group B and C rabbits respectively.

### Parameters studied

Following parameters were studied:

#### Body weight

Body weight was determined by an electric balance (SHIMADZU BX-3000 Japan). The rabbits were weighted on very first day of experiment to have initial body weight and then on weekly bases to calculate the body weight changes till the termination of the experiment. The weight changing was calculated by previous body weight from new body weight.

### Blood collection

The blood samples were collected every 7<sup>th</sup> day (one week) from cephalous vein of rabbits with sterile disposable syringe after rubbing vein with xylene, so that the veins become swollen. The needle of 24 gauges was used for sucking the blood, which was inserted at the angle of 45. 2ml blood (each time) was collected in test tubes for serological studies (RFT).

### Serum separation

The serum was isolated according to the procedure reported in manual of basic Techniques for healthy laboratory (WHO-Geneva, 1980).

- The sampling tubes containing blood were allowed to clot at room temperature for about 2-3 hours.
- The clotted blood was then centrifuged at 4000 rpm for about 15 minutes.
- After centrifugation the cellular portion of blood along with fibrinogen was accumulated in bottom of centrifuge tube and light straw color serum was then separated.
- The serum was then transferred to sterile eppendroff tubes with the help of pasture's pipette.
- Finally eppendroff tubes were capped and labeled for storage.
- The serum samples of each rabbit was collected and stored separately.

### Storage of serum

The labeled sampling eppendroff tubes containing serum were stored in the freezer compartment of the refrigerator at -8°C to -10°C until they were subjected to the analysis. Serum once separated could be stored for fairly long time by freezing.

### Kidney Function Test (RFT)

The levels of serum creatinine and urea were determined based on the following the principles.

#### Creatinine

The end product of reaction of creatinine with alkaline picrate is an orange yellow color (Jaffe's reaction). There is direct proportion between absorbance of orange yellow color and amount of creatinine in the sample. The absorbance is measured at 510nm (Patil *et al.*, 2014). The amount of creatinine is determined in mg/dl.

#### Urea

The end products of hydrolysis of urea in the presence of water and urease are ammonia and carbon dioxide. Ammonia reacts with ketoglutarate in the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH) to produce L-glutamate. Adenosine diphosphate (ADP) is used as an activator and stabilizer of GLDH. The results of reaction are determined by measuring the decrease in absorbance at 340 nm, as NADH is oxidized to NAD. The value is expressed in mg/dl (Patil *et al.*, 2014).

#### Statistical analysis:

Statistical analysis was performed through general linear models. Univariate ANOVA was applied for the

parameters (Scheffe, 1959) at 95% confidence interval. The significant values were expressed by asterisk ( $p < 0.05$ ). The data was expressed as mean  $\pm$  S.D.

## RESULTS

Influence of Sodium selenite at dose of 2mg/kg in group "B" was analyzed. The mean values of Creatinine in experimental group B on 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>th</sup>, and 28<sup>th</sup> days were  $1.26 \pm 0.207$ ,  $2.74 \pm 0.26$ ,  $3.22 \pm 0.2607$  and  $4.34 \pm 0.2885$  mg/dl (Table 1). The level of Creatinine increased in group "B" as compared to control at the end of 28<sup>th</sup> day of the experiment (Figure 1). The value of F was 10.746 showed highly significant results. The level of significance was 0.017 that is less than 0.05.

**Table 1. Effect of sodium selenite at dose 2mg/kg on Creatinine mg/dl level in rabbit**

Sr.No.	Sampling Day	Creatinine mg/dl level (Mean $\pm$ SD)	
		Control group A	Experimental group B
1	7 <sup>th</sup> day	0.9 $\pm$ 0.27	1.26 $\pm$ 0.207*
2	14 <sup>th</sup> day	0.72 $\pm$ 0.17	2.74 $\pm$ 0.26*
3	21 <sup>st</sup> day	0.86 $\pm$ 0.27	3.22 $\pm$ 0.2607*
4	28 <sup>th</sup> day	0.68 $\pm$ 0.19	4.34 $\pm$ 0.2885*

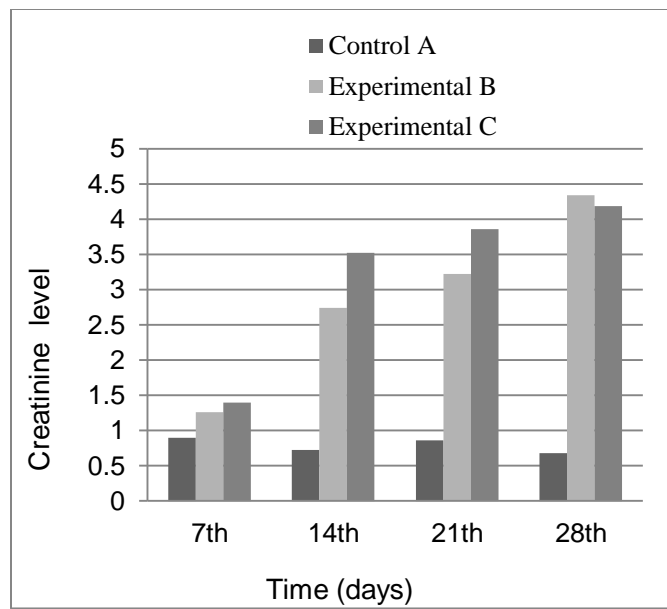
Values with \* are significant.

Influence of Sodium selenite at dose of 3mg/kg in group "C" was analyzed. The mean values of Creatinine level in experimental group C on 7<sup>st</sup>, 14<sup>th</sup>, 21<sup>th</sup>, and 28<sup>th</sup> days were  $1.4 \pm 0.26$ ,  $3.52 \pm 0.16$ ,  $3.86 \pm 0.12$  and  $4.18 \pm 0.22$  mg/dl (Table 2). The level of Creatinine was increased in group "C" as compared to control at the end of 28<sup>th</sup> day of the experiment (Figure 1). The value of F was 24.749 showed highly significant results. The level of significance was 0.003 that is less than 0.05.

**Table 2. Effect of sodium selenite at dose 3mg/kg on Creatinine mg/dl level in rabbit**

Sr.No	Sampling Day	Creatinine mg/dl level (Mean $\pm$ SD)	
		Control group A	Experimental group C
1	7 <sup>th</sup> day	0.9 $\pm$ 0.27	1.4 $\pm$ 0.26*
2	14 <sup>th</sup> day	0.72 $\pm$ 0.17	3.52 $\pm$ 0.16*
3	21 <sup>st</sup> day	0.86 $\pm$ 0.27	3.86 $\pm$ 0.12*
4	28 <sup>th</sup> day	0.68 $\pm$ 0.19	4.18 $\pm$ 0.22*

Values with \* are significant.



**Fig. 1. Graph showing Creatinine level of Control group as well as group medicated with sodium selenite at dose 2, & 3 mg/kg in rabbit.**

Influence of Sodium selenite at dose of 2mg/kg in group "B" was analyzed. The mean values of urea level in experimental group B on 7<sup>st</sup>, 14<sup>th</sup>, 21<sup>th</sup>, and 28<sup>th</sup> days were  $40 \pm 0.79$ ,  $44.6 \pm 1.92$ ,  $52 \pm 2.76$  and  $57.4 \pm 2.79$  mg/dl (Table 3). The level of urea was increased in group "B" as compared to control at the end of 28<sup>th</sup> day of the experiment (Figure 2). The value of F was 7.130 showed highly significant results. The value of significance was 0.037, is less than 0.05.

**Table 3. Effect of sodium selenite at dose 2mg/kg on Urea mg/dl level in rabbit**

Sr.No	Sampling Day	Urea mg/dl level (Mean $\pm$ SD)	
		Control group A	Experimental group B
1	7 <sup>th</sup> day	40 $\pm$ 2.76	40 $\pm$ 0.79*
2	14 <sup>th</sup> day	38.2 $\pm$ 2.07	44.6 $\pm$ 1.92*
3	21 <sup>st</sup> day	37.2 $\pm$ 2.07	52 $\pm$ 2.76*
4	28 <sup>th</sup> day	36.6 $\pm$ 2.28	57.4 $\pm$ 2.79*

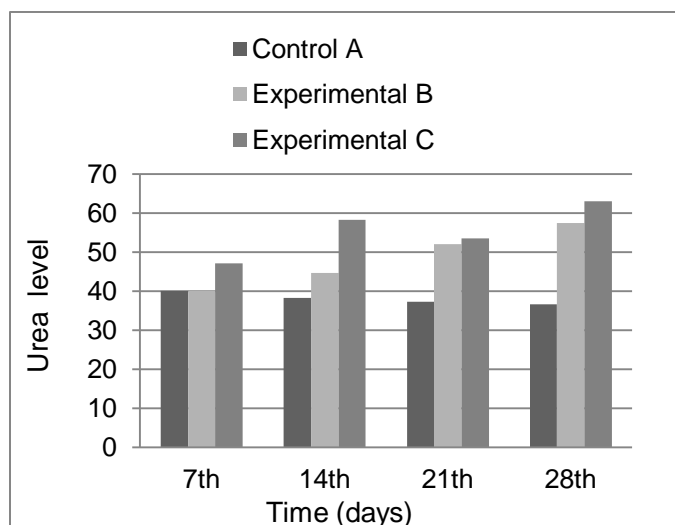
Values with \* are significant.

Influence of Sodium selenite at dose of 3mg/kg in group "C" was analyzed. The values of urea level in experimental group C on 7<sup>st</sup>, 14<sup>th</sup>, 21<sup>th</sup>, and 28<sup>th</sup> days were  $47 \pm 3.22$ ,  $58.2 \pm 4.36$ ,  $53.4 \pm 2.19$  and  $63 \pm 2.42$  mg/dl (Table 4). The level of urea was increased in group "C" as compared to control at the end of 28<sup>th</sup> day of the experiment (Figure 2). The value of F was 24.749 showed highly significant results. The value of significance was 0.003, is less than 0.05.

**Table 4. Effect of sodium selenite at dose 3mg/kg on Urea mg/dl level in rabbit**

Sr.No	Sampling Day	Urea mg/dl level (Mean $\pm$ SD)	
		Control group A	Experimental group C
1	7 <sup>th</sup> day	40 $\pm$ 2.76	47 $\pm$ 3.22*
2	14 <sup>th</sup> day	38.2 $\pm$ 2.07	58.2 $\pm$ 4.36*
3	21 <sup>st</sup> day	37.2 $\pm$ 2.07	53.4 $\pm$ 2.19*
4	28 <sup>th</sup> day	36.6 $\pm$ 2.28	63 $\pm$ 0.2885*

Values with \* are significant.



**Fig.2. Graph showing Urea level of Control group as well as group medicated with Sodium selenite at dose 2, & 3 mg/kg in rabbit.**

## DISCUSSION

In poultry and livestock Sodium selenite feed are used to support growth, and its forms are present in the waste chemical disposal sites which are indirectly reached to the ground or drinking water.

Serum concentrations of urea nitrogen and creatinine are traditional screening tests used to evaluate renal function. Our results showed that higher level of sodium selenite in the diet of experimental rabbits is responsible for the increase in creatinine in blood serum. The highest amount of creatinine was found at 28<sup>th</sup> day. The presence of higher amount of creatinine in blood serum is responsible for nephrotoxicity. Previous studies also demonstrated raised amount of serum creatinine after feeding rabbits with selium supplemented forage. The serum creatinine showed significant difference ( $P < 0.05$ ) among treatment (Saleh *et al.*, 2015).

Creatinine test has advantages over urea as a renal function test because it is inactive metabolically. Creatinine is derived entirely from endogenous metabolism. Its

excretion is independent of diet (Krishnaswamy and Lukose, 2015). When renal function fails, the serum Creatinine rises. The Creatinine clearance is by far the most sensitive regular clinical test of renal functioning available today. Normal values of Creatinine vary depending on the method and specimen used (Rule *et al.*, 2004). Creatinine ratio can be used to draw additional clinical data about the status of renal function (Levey *et al.*, 2006). In present study the level of Creatinine concentration was significantly higher in case of Group B and C as compared to Group A indicating renal toxicity. The results of previous studies suggested that selenium was more toxic to female rats than to males (Fitzhugh *et al.*, 1944). Schroeder (1967) observed a higher number of deaths in male Long-Evans rats than in females receiving 2 ppm selenium as sodium selenite in the drinking water. Like kidney function tests, variable values of serum parameters for liver function tests are responsible for liver disorders in humans (Muhammad *et al.*, 2013; Toor *et al.*, 2016).

Our results showed that higher level of sodium selenite in the diet of experimental rabbits is responsible for the increase in urea in blood serum. The highest amount of urea was found at 28<sup>th</sup> day. The presence of higher amount of urea in blood serum is responsible for nephrotoxicity. Previous studies also demonstrated raised amount of serum urea after feeding rabbits with selium supplemented forage (Saleh *et al.*, 2015). The serum urea values were within the normal range reported previously (Njidda and Isidahomen, 2011; Njidda and Isidahomen, 2010) who fed sesame seed meal and grasshopper meal to rabbit. Duncan and Prasse (1986) reported lower serum urea values that are against our findings. In many renal diseases, the glomerular filtration rates of two kidney falls considerably below normal resulting in decreased excretion of urea (Guyton 1991). However, the body continues to form large quantities of urea which means the urea will progressively collect in the body fluids until its plasma concentration rises very high. Higher concentrations of urea and creatinine in blood are responsible for renal toxicity.

## CONCLUSIONS

Observation of current study describes that sodium selenite changes the level of the serum Creatinine and Urea in Rabbits (especially in the case when affected by the liver). The toxicity is higher when administered by the high dose of sodium selenite in the diet as compared to the low dose.

## ACKNOWLEDGMENTS

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## Conflict Of Interest

The authors declare that they have no competing interests.

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