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Biochemical Evaluation of Selenium Toxicity on Rats

KEYWORDS: sodium selenite, lipid peroxidation, antioxidant enzymes, rats

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ABSTRACT The present study was undertaken to investigate the possible toxicological disorders as a result of oral administration of ¼ LD50, ½ LD50, LD50 of sodium selenite as well as LD50 + Vitamin E into male albino rats. Blood samples were collected 6, 12, 24 and 30 hours post administration for the determination of lipid peroxidation content and the activities of catalase, Cu/Zn-SOD, GPx enzymes. The obtained results were discussed in details. It is concluded that catalase was the most affected enzyme with sodium selenite administration with respect to lipid peroxidation and GPx was the most affected among antioxidant enzymes. It is also concluded that vitamin E alleviates the adverse effect of sodium selenite administration in most measured parameters at different time intervals. Finally, some measured parameters were dose-dependent at certain selenite dose administrations and were time-dependent at certain time intervals.

INTRODUCTION

Selenium is a naturally occurring trace element that is widely but unevenly distributed in the earth's crust and is commonly found in sedimentary rocks. When these rocks change to soils, selenium combines with oxygen to form several compounds, the most common of which are sodium selenite and sodium selenate. The amount of selenium in soil, which varies by region, determines the amount of selenium in the plant foods that are grown in that soil. Selenium in beef, white bread, pork, chicken, and eggs accounted for about 50 % of the total selenium consumed in the diet [1].

Selenium (Se), has multiple biological activities, which depend on the level of Se intake. Relatively low Se intakes determine the expression of selenoenzymes in which it serves as an essential constituent. Higher intakes have been shown to have anti-tumorigenic potential; and very high Se intakes can produce adverse effects. This hierarchy of biological activities calls for biomarkers informative at different levels of Se exposure [2].

Selenium is harmful to humans and animals when eaten in amounts much lower or higher than the amounts needed for good nutrition. Selenium deficiency in animals results in effects such as growth retardation, reproductive failure and degenerative organ changes. In humans, selenium deficiency is thought to be involved in Keshan disease, and Kashin-Beck disease, while the selenium overdose can cause a condition known as selenosis or selenium toxicity [3]. Because of selenite possesses substantial bioavailability for plants and animals, it is necessary to explore its mechanism of actions, and its physiological and behavioral dangers on albino rats in the present study.

Vitamin E is also an important antioxidant in preventing cellular damages caused due to lipid peroxidation. Vitamin E and Selenium are necessary nutrients that are important ingredients of the antioxidant system, to be responsible for defense of tissues and cells. Selenium, a constituent of enzyme Glutathione, along with vitamin E assists as a biological antioxidant and maintains cellular consistency [4][5].

The present study is undertaken to clarify the following questions:

Is there any significant difference between untreated control and selenite-treated rats? Is the difference dose-dependent? Is the difference time-dependent? Which exposure time was more affected with selenite treatments? Which antioxidant is more affected with selenite, catalase, Cu/Zn SOD or GPx? Does vitamin E have a protective effect on selenite toxicity?

In order to answer the above questions, the present study is subjected to evaluate lipid peroxidation content and endogenous antioxidants such as the activities of catalase, Cu/Zn-SOD and GPx.

MATERIALS & METHODS

1- Materials

Chemicals:

Sodium Selenite ($Na_2SeO_3.5H_2O$), Vitamin E: DL- -Tocopherol acetate, Lipid Peroxidation (Malondialdehyde), Catalase (Cat), Speroxide Dismutase (SOD), Glutathione Peroxidase (GPx) was purchased from Sigma and Biodiagnostic.

Experimental animals:

Adult Male Wistar Albino rats (150 – 170 gm) were kept in cages under standard conditions; i.e. a well ventilated room (25±4 °C), relative humidity 30-70%, and 12 hours controlled regimen of fluorescent light (light for 12 hours, and dark for 12 hours) at the animal house of King Abdull Aziz University. Rats were regularly fed on standard diet ad-libitum.

2- Methods:

Lethality Assay: The LD_{50} of sodium selenite was measured [6] and estimated to be 18.7 mg/kg body weight.

Study Design: 126 male Wistar Albino rats were adapted to the environment for one week and then they were randomly divided into control group (6 rats), selenite-treated groups (96 rats) as well as vitamin E and LD_{50} selenite group (24 rats).

*Control group: 6 rats were orally administered with distilled water as a vehicle after 24 hours of fasting and collect blood after 6, 12, 24 and 30 hours.

*Selenite-Treated groups: Selenite-treated groups (120 rats) were subdivided into 4 subgroups. 1/4 LD_{50}, 1/2 LD_{50}, LD_{50} and

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LD_{50} + Vit E Blood samples were collected after 6 hours, 12 hours, 24 hours and 30 hours of administration from the 1st, 2nd, 3rd and 4th subgroups respectively.

Blood samples were collected [7] for determination of lipid peroxidation as malondialdehyde content in the blood serum and endogenous antioxidants such as the activities of enzymes catalase, Cu/Zn-SOD and Gpx.

Statistical analysis: Data were using SPSS software[8]. One-way ANOVA was carried out to find if there is any significant difference between control and all treated groups. The two-factor ANOVA with the type III sum-of-squares method by means of multi-variant general liner models (GLM) were used to investigate the effect of exposure time to three doses of selenium and their interaction on biochemical parameters. Pearson's correlation test was used to evaluate the linear relationship between oxidative stress biomarkers (lipid peroxidation content) and antioxidants.

RESULTS & DISCUSSION

Biochemical examinations

1- Lipid peroxidation content

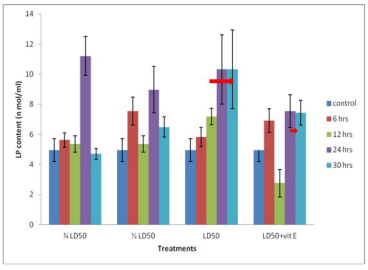

Fig. (1): Effect of different oral doses administration of sodium selenite on Lipid Peroxidation (LP) content (n mol/ml) of rat at different time intervals

The result of the present study showed that treatment with sodium selenite caused a general significant elevation of lipid peroxidation content in all exposure times (6, 12, 24 and 30 hours) and in all exposure doses post administration. The most potent effect of sodium selenite on lipid peroxidation content observed at LD50 treatment after 24 and 30 hours of exposure, (fig. 1). The data showed that vitamin E significantly alleviates the toxic effect of sodium selenite after 30 hours of treatment. . The data also showed that lipid peroxidation content after sodium selenite treatment was dose-dependent (fig. 2) and time-dependent (fig. 3).

Most researchers actually had believed that lipid peroxidation increase dramatically with increased work, exercise, infection, or disease [9] ; [10].

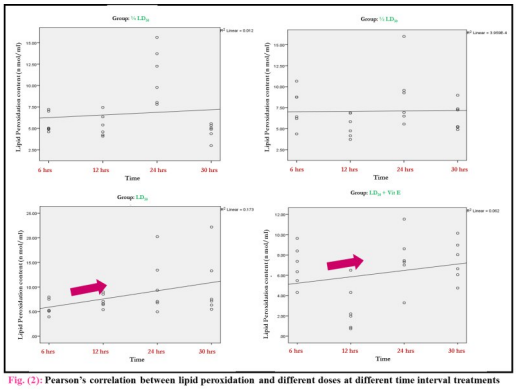

Fig. (2): Pearson's correlation between lipid peroxidation and different doses at different time interval treatments

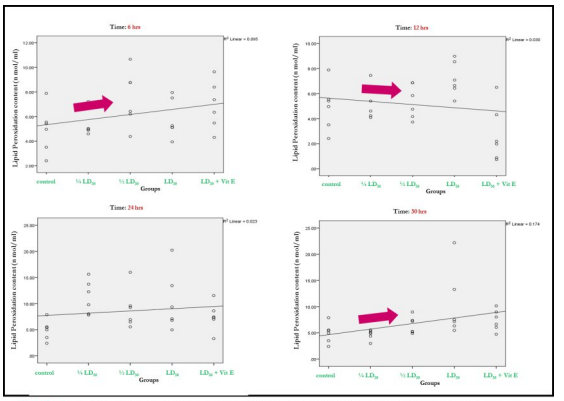

Fig. (3): Pearson's correlation between lipid peroxidation and different time intervals at different dose treatments

2- Catalase activity

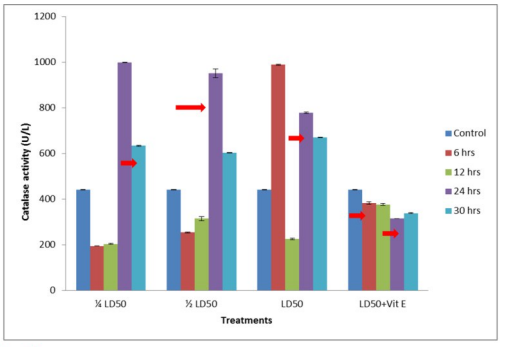

Fig. (4): Effect of different oral doses administration of sodium selenite on **Catalase** activity (U/L) of rat at different time intervals

The results of the present study showed that treatment with sodium selenite caused a significant decrease of catalase activity at exposure of ½ LD50 dose at 6 and 12 hours post administration. The most potent effect of sodium selenite on catalase activity observed after 6 hours of treatment, (fig. 4). The data showed that vitamin E significantly alleviates the toxic effect of sodium selenite after 6 hours and 24 hours of treatment. The data also showed that catalase activity after sodium selenite treatment was dose-dependent (fig. 5) and time-dependent (fig.6).

The present results could be interpreted that ½ LD50 dose of sodium selenite caused the formation of free radicals (superoxide radical) that lead to formation of H2O2 which converted to H2O and O2 by catalase, that may be decreased in activity during this reaction[11].

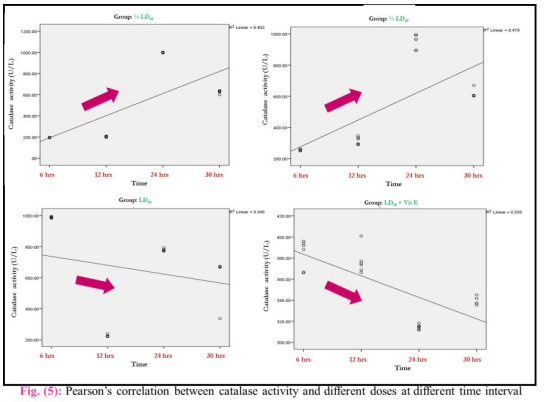

Fig. (5): Pearson's correlation between catalase activity and different doses at different time interval treatments

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

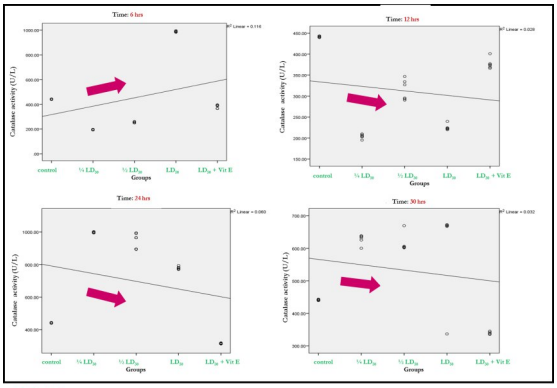

Fig. (6): Pearson's correlation between catalase activity and different time intervals at different dose treatments

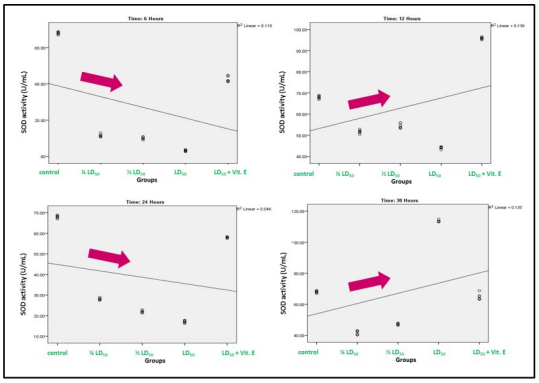

Fig. (9): Pearson's correlation between SOD activity and different time intervals at different dose treatments

3- Superoxide Dismutase (SOD) activity

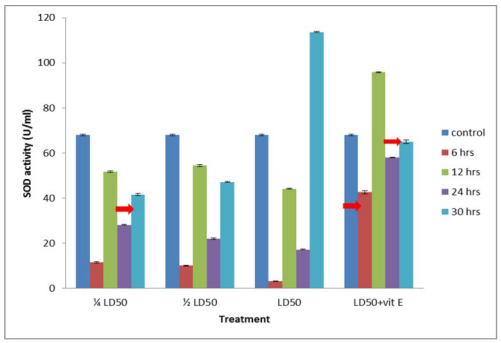

Fig. (7): Effect of different oral doses administration of sodium selenite on Superoxide Dismutase (**SOD**) activity (U/ml) of rat at different time intervals

The results of the present study showed that treatment with sodium selenite caused a significant decrease of SOD activity in all exposure doses at all times of administration (fig.7). The most potent effect of sodium selenite on SOD activity observed after 30 hours at ¼ LD50 of treatment. The data showed that vitamin E significantly alleviates the toxic effect of sodium selenite after 6, 24 and 30 hours of treatment. The data also showed that SOD activity after sodium selenite treatment was dose-dependent (fig.8) and time-dependent (fig.9). This result could be interpreted that the decreased Cu/Zn SOD activity was induced to sweep away free radicals occurred after sodium selenite administration [11, 12, 13]. The decrease in SOD activity in the present work could be attributed to the loss of copper and zinc which are essential for the activity of enzyme or to reactive oxygen species (ROS) induced inactivation of enzyme proteins[14,15,16]

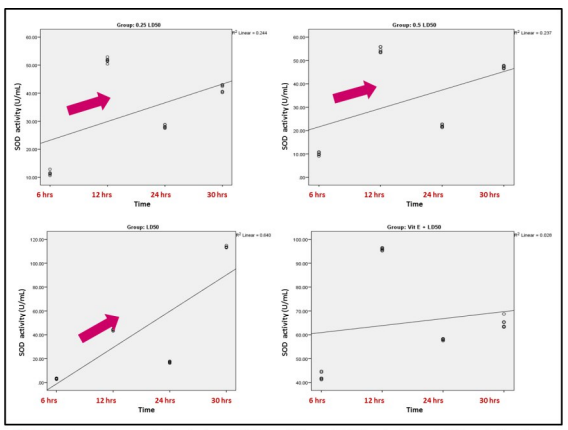

Fig. (8): Pearson's correlation between SOD activity and different doses at different time interval treatments

4- Glutathione peroxidase (GPX) activity

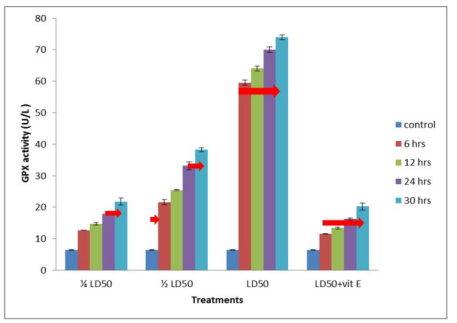

Fig. (10): Effect of different oral doses administration as sodium selenite on Glutathion Peroxidase (**GPx**) activity (U/L)of rat at different time intervals

The data showed that GPX activity was significantly increased after 12, 24 and 30 hours post ¼ LD50 sodium selenite administration. The data also showed significant increase after 6, 24 and 30 hours post ½ LD50 sodium selenite administration with percentage of changes from control. Also the data showed significant increases in GPX activity after LD50 sodium selenite administration in all exposure times. The data also showed that GPX activity was significantly increased after 6 and 24 hours post LD50 + Vit. E sodium selenite administration when compared to LD50 sodium selenite administration.

The increase in GPx activity in the present work (fig.10) could be attributed to the increase in Malondialdehyde (MDA) content [17]. The data also showed that GPx activity after sodium selenite treatment was dose-dependent (fig.11) and time-dependent (fig.12).

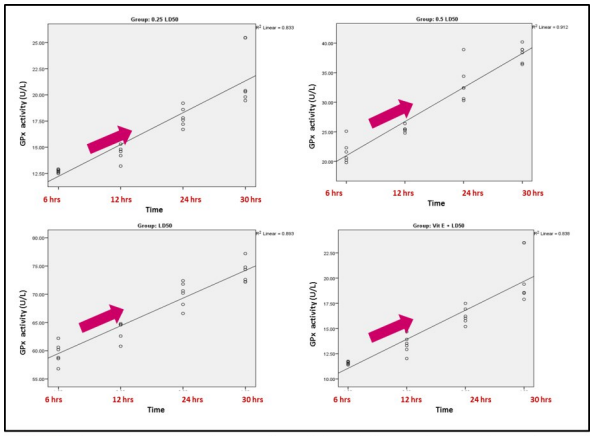

Fig. (11): Pearson's correlation between GPx activity and different doses at different time interval treatments

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

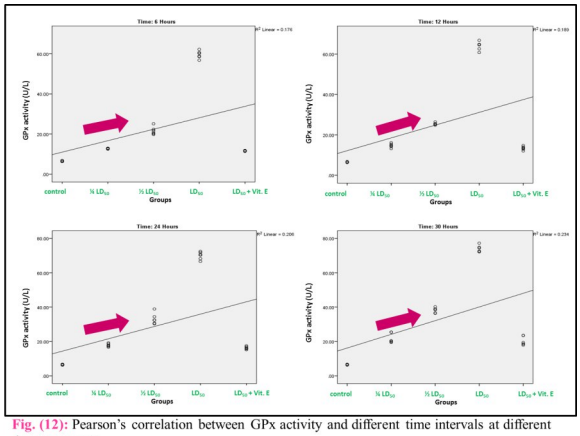

Fig. (12): Pearson's correlation between GPx activity and different time intervals at different dose treatments

Table (1): Change in lipid peroxidation as a biomarker and antioxidant enzymes after ¼ **LD_{50}** of sodium selenite administration at different time intervals (using One-Way ANOVA)

Parameters \ Times	control	6 Hours	12 Hours	24 Hours	30 Hours	$F_{1,5}$
Lipid Peroxidation content (MDA n mol/ml)	4.95 ± 0.77 [a]	5.62 ± 0.48	5.35 ± 0.54	11.20 ±1.30	4.69 ± 0.38	15.48*
Catalase activity (U/ml)	441.31 ± 0.67	195.35 ± 0.74	203.92 ± 2.07	998.07 ± 1.21	633.77 ± 2.39	55633.42*
Cu/Zn SOD activity (U/ml)	68.00 ± 0.28	11.51 ± 0.31	51.68 ±0.33	28.13 ± 0.23	41.60 ± 0.52	2289.45*
Glutathione peroxidase (U/L)	6.47 ± 0.07	12.73 ±0.07	14.70 ±0.40	17.85 ± 0.37	21.81 ± 1.16	38.04*

(a) Represents mean value ± standard error of 6 rat /group.

(*) Represents *F* ratio is significant at $p<0.05$

Table (2): Change in lipid peroxidation as a biomarker and antioxidant enzymes after ½ **LD_{50}** of sodium selenite administration at different time intervals (using One-Way ANOVA)

Parameters \ Times	control	6 Hours	12 Hours	24 Hours	30 Hours	$F_{1,5}$
Lipid Peroxidation content (MDA n mol/ml)	4.95 ± 0.77 [a]	7.53 ± 0.93	5.36 ± 0.55	8.97 ±1.55	6.48 ± 0.67	2.35
Catalase activity (U/ml)	441.31 ± 0.67	254.09 ± 2.03	314.46 ± 9.98	950.71 ± 1.85	602.85 ± 0.81	910.18*
Cu/Zn SOD activity (U/ml)	68.00 ± 0.28	10.04 ± 0.27	54.47 ± 0.47	21.98 ± 0.25	47.15 ± 0.23	4334.41*
Glutathione peroxidase (U/L)	6.47 ±0.07	21.60 ± 0.79	25.46 ± 0.22	33.18 ±1.29	38.24 ± 0.60	82.66*

(a) Represents mean value ± standard error of 6 rat /group.

(*) Represents *F* ratio is significant at $p<0.05$

Table (3): Change in lipid peroxidation as a biomarker and antioxidant enzymes after **LD_{50}** of sodium selenite administration at different time intervals (using One-Way ANOVA)

Parameters \ Times	control	6 Hours	12 Hours	24 Hours	30 Hours	$F_{1,5}$
Lipid Peroxidation content (MDA n mol/ml)	4.95 ± 0.77 [a]	5.81 ± 0.64	7.18 ± 0.55	10.31 ±2.31	10.32 ±2.63	1.596
Catalase activity (U/ml)	441.31 ± 0.67	988.51 ± 1.84	225.28 ± 2.94	778.30 ± 3.54	669.83 ± 0.78	16446.89*
Cu/Zn SOD activity (U/ml)	68.00 ± 0.28	3.15 ± 0.16	44.08 ± 0.21	17.13 ± 0.25	113.64 ±0.26	47578.17*
Glutathione peroxidase (U/ml)	6.47 ± 0.07	59.53 ±0.77	64.03 ±0.84	69.97 ± 0.90	73.90 ± 0.80	58.55*

(a) Represents mean value ± standard error of 6 rat /group.

(*) Represents *F* ratio is significant at $p<0.05$

Table (4): Change in lipid peroxidation as a biomarker and antioxidant enzymes after **LD_{50}+Vit. E** of sodium selenite administration at different time intervals (using One-Way ANOVA)

Parameters \ Times	control	6 Hours	12 Hours	24 Hours	30 Hours	$F_{1,5}$
Lipid Peroxidation content (MDA n mol/ml)	4.95 ± 0.77 [a]	6.92 ± 0.80	2.76 ± 0.91	7.53 ±1.09	7.43 ± 0.81	6.29*
Catalase activity (U/ml)	441.31 ± 0.67	383.72 ± 5.63	376.88 ± 5.09	314.47 ± 0.99	338.87 ± 1.51	69.95*
Cu/Zn SOD activity (U/ml)	68.00 ± 0.28	42.55 ± 0.63	95.83 ± 0.21	58.00 ±0.14	64.91 ±0.86	1668.09*
Glutathione peroxidase (U/L)	6.47 ± 0.07	11.60 ± 0.06	13.40 ± 0.37	16.27 ± 0.34	20.23±1.05	41.60*

(a) Represents mean value ± standard error of 6 rat /group.

(*) Represents *F* ratio is significant at $p<0.05$

Tables (1, 2, 3 and 4) summarize the biochemical measurements after ¼ LD50, ½ LD50, LD50 and LD50+Vit. E administration of sodium selenite for lipid peroxidation and antioxidant enzymes (catalase, SOD and GPX) in all exposure times (6, 12, 24 and 30 hours). One-way ANOVA for selenite treatment in all times revealed significant difference for activity with F1, 5 value equals 38.04 after ¼ LD50 administration, 82.66 after ½ LD50 administration, 58.55 after LD50 administration and 41.60 LD50 + Vit. E administration.

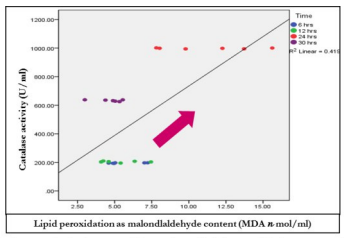

Fig. (13): The correlation analysis between biomarker (lipid peroxidation content) and catalase activity after ¼ **LD_{50}** sodium selenite administration

The correlation between catalase activity and lipid peroxidation content after sodium selenite administration are presented in (fig.13) and showed that there was an inverse relationship between them after LD50 administration, i.e. as lipid peroxidation content increased, catalase activity decreased[18]. On the other hand, the table showed that there is a significant directly (or positive) correlation between them at ¼ LD50 exposure.

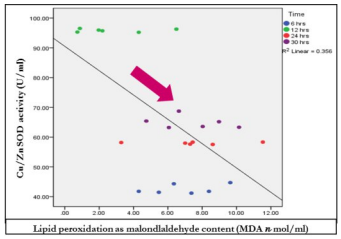

Fig. (14): The correlation analysis between biomarker (lipid peroxidation content) and SOD activity after **LD_{50} + vit. E** sodium selenite administration

The correlations between Cu/Zn SOD activity and lipid peroxidation after sodium selenite administration was presented in (fig.14). These correlations showed that there was a regular succession relationship between them, i.e. as lipid peroxidation content increased, Cu/Zn SOD activity decrease. These results can be interpreted that increased lipid peroxidation contents was a biomarker for high free radicals concentration and decrease in SOD activity could be an adaptive response of this enzyme to increased production of free radical formation followed sodium selenite dose administrations[19, 20].

Table (5): Two-way ANOVA of **oxidative stress** parameters after sodium selenite administration

Parameters	Dose*time	
	Sig.	$F_{(17,36)}$
Lipid Peroxidation content (MDA µmol/ml)	.015*	2.249
Catalase activity (U/ml)	.000*	2048.80
Cu/Zn SOD activity (U/ml)	.000*	3426.29
Glutathione peroxidase (U/L)	.000*	19.98

(*) represents *F* ratio in significant at $p < 0.05$

Table (5) summarizes the two-way ANOVA model (treatment + time) for oxidative stress parameters measured in all times (6, 12, 24 and 30 hours). The two-way ANOVA for lipid peroxidation content, catalase activity, SOD activity and GPX activity revealed a highly significant difference.

It is concluded that catalase was the most affected enzyme with sodium selenite administration with respect to lipid peroxidation and GPx was the most affected among

Volume : 6 | Issue : 11 | November 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

antioxidant enzymes. It is also concluded that vitamin E alleviates the adverse effect of sodium selenite administration in most measured parameters at different time intervals. Finally, some measured parameters were dose-dependent at certain selenite dose administrations and were time-dependent at certain time intervals.

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