Journal of Applied Pharmaceutical Science Vol. 3 (06), pp. 136-145, June, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3623
ISSN 2231-3354

Assessment of the Safety of Chronic Administration of Selenium Enriched Yeast

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ARTICLE INFO

Article history:
Received on: 09/05/2013
Revised on: 25/05/2013
Accepted on: 11/06/2013
Available online: 27/06/2013

Key words:
Selenium, *Saccharomyces cerevisiae*, Seleinum - enrichment yeast and rats.

ABSTRACT

This study aimed to achieve a balance between selenium (Se) incorporation and optimal growth of yeast (*Saccharomyces cerevisiae*) cells, and to assess the safety of administration of selenium-enriched yeast (Se-Y) in comparison with inorganic sodium selenite in experimental animals. Se as sodium selenite was incorporated into yeast cells in different concentrations (19, 39 and 57 µM Se), then dry yeast biomass, yeast cell viability and Se content of the yeast were evaluated. Acute toxicity of Se, yeast (*Saccharomyces cerevisiae*) and Se-Y were determined. In chronic toxicity study, one hundred and eight (male and female) rats were classified into 10 groups. Group (1) was control. Groups 2, 3 and were given three dose levels of Se 3.8, 1.91 and 0.95 mg/kg/day respectively for 3 months. Groups 5, 6 and 7 were given three dose levels of yeast 250, 125 and 66.5 mg/kg/day respectively for 3 months. Groups 8, 9 and 10 were given three dose levels of Se-Y 250, 125 and 66.5 mg/kg/day respectively for 3 months. Heamoglobin (Hb) concentration, white blood cells (WBCs) count, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activity, urea and creatinin levels were determined. Moreover, histopathological examination of liver and kidney tissue sections were evaluated. With the increase of Se concentration added to the fermentation medium, the significant increase in the selenium content in yeast cells was recorded. While, the increased Se incorporation in the yeast caused a significant decrease in dry yeast biomass and cell viability as compared to blank. The present results also showed that animals administered yeast at different doses for 3 months displayed nearly no changes in the studied biochemical parameters. Administration of Se or Se-Y in high and intermediate doses significantly decreased Hb level and WBCs counts, while they caused significant increase in ALT, AST, ALP activity as well as urea and creatinin levels. Administration of Se or Se-Y in low dose exhibited nearly no changes in AST activity while they caused significant increase in ALT and ALP activities. Moreover, administration of Se in low dose caused significant increase in urea and creatinin levels in both male and female rats. These results are well documented by histopathological findings. The present study indicated that 19 µM Se could be the proper concentration for achieving the optimal growth of yeast cells. Additionally, this study asserted the safety of chronic administration of Se-Y compared with inorganic Se.

INTRODUCTION

Life would not be possible without a large number of trace elements, each serving critical roles in metabolism and function (Rahil-Khazen *et al*., 2002). Selenium (Se) is an essential trace element for humans, which improves the activity of the selenoenzyme. It is present in the active center of glutathione peroxidase (GPx), an antioxidant enzyme, which protects lipid membranes and macromolecules from oxidative damage (Heikal *et al*., 2012). Se has the ability to counteract free radicls and protect the structure and function of proteins, DNA and chromosomes against oxidation injury (Akhtar *et al*., 2009). Thus, a dietary deficency in Se can increase the sensitivity of a living system to oxidative stress as the protein and mRNA levels for several selenoproteins are down- regulated dramatically by Se deficiency (Rains and Sunde, 2011). Brewer's and baker's yeast *(Saccharomyces cerevisiae)* has been used in classical food fermentation applications (beer, bread, yeast extract/vitamins, wine, saké, and distilled spirits) (Beudeker *et al*., 1990). Hartwell *et al*. (1997) have pioneered a noval approche in which the yeast *Saccharomyces cerevisiae* is used to discover compounds with powerful chemotherapeutic potential.

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Remarkably, studies on *Saccharomyces cerevisiae* as a model system have provided invaluable insights into the action of many toxins or drugs and compounds with quite specific activities in both mammals and fungi (Cardenas *et al*., 1999).

The use of selenized yeast as enriched selenium supplements in human nutrition has become a topic of increasing interest over the last decade. Due to nutritional benefits, selenium-enriched yeast (Se-Y) is a common form of Se used to supplement the dietary intake of this important trace mineral (Rayman, 2004). The present study was undertaken to achieve a balance between Se incorporation and optimal growth and viability of yeast cells. Additionally, the study aimed at assessing the safety of chronic administration of selenium as selenium-enriched yeast in comparison with selenium as sodium selenite form in experimental animals.

MATERIALS AND METHODS

Yeast strain, *S. cerevisiae* was obtained from Sugar and Integrated Industrial Company (Egypt). Sodium selenite was obtained from PROLABO Co. (France). Yeast extract; peptone, dextrose and agar were obtained from Sigma Chemical Co. (USA).

Preparation of yeast culture

YEPD medium used in the present study contained the following components: 3g yeast extract, 10g peptone and 20g dextrose in one liter of distilled water with final pH 4.5. The yeast strain was maintained on the YEPD agar slants (2.5% agar). Then yeast, *S. cerevisiae*, was inoculated from agar slants in 10 ml of YEPD medium and incubated overnight at 30° C in a shaker incubator at 150 rpm. This culture was streaked on agar plates and incubated at 30° C for 24-48 hours. Further, one colony was inoculated in fresh YEPD medium and incubated overnight at 30° C in a shaker incubator at 150 rpm (Kaur and Bansal, 2006).

Selenium supplementation to culture

Se in inorganic form as sodium selenite (Na_2SeO_3) was used, from 25 mM sodium selenite stock solution 19, 39 and 57 µM Se concentrations were prepared in different 20 ml aliquots of yeast culture media. Media without addition of Se was used as blank. These Media with different Se concentrations were inoculated with 100 µl of overnight culture and incubated at 30°C for overnight growth on shaker (Kaur and Bansal, 2006). After incubation, Se was estimated in cell pellet from 20 ml overnight culture. Dry matter of yeast biomass was determined by drying the yeast biomass at 105 °C to a constant weight after centrifuging 5 ml of samples at 4000 rpm for 10 min on a portable centrifuge.

Determination of yeast cell viability

In order to determine the viability of yeast cells, 2 ml of post-culturing liquid was centrifuged and then decanted. The remaining yeast was washed with distilled water, decanted, and topped up with water to the initial volume of 2 ml. After thorough stirring, one drop of the solution was placed in a Thom chamber, and a drop of 0.01% solution of methylene blue was added to the sample to dye dead cells. In the resultant preparation, a count was taken of living cells (non-dyed) and of dead cells (dyed blue). The percentage of dead cells was a mean value for 16 fields calculated according to the following formula:

Percent of dead cells = (number of dead cells /sum of dead and living cells) x 100% (Urszula and Jamroz, 2007).

Determination of selenium concentration

Se concentration in the mineralized yeast was estimated using atomic absorption spectrophotometry method as follows: portions of 100 mg of lyophilized selenium yeast were weighed into glass thimbles, then 5 ml of HNO_3-$HClO_4$ (3+1 v/v) mixture was added, and the sample mixture was left overnight. The following day, the samples were heated in a heating block at 50^{o}C for 1 h, at 70^{o}C for 6 h, and at 125^{o}C for 12 h. After cooling, solutions were transferred to measuring flasks of 25 ml in volume and topped up with distilled water. Then, Se was determined using atomic absorption spectrophotometry, with the flameless technique, using a graphite cuvette in a Varian Spectra AA-880 apparatus (Urszula and Jamroz, 2007).

Toxicological studies

Male and female Sprague Dawley rats weighing 120-150 g were used in the current study. The animals fed standard laboratory pellet diet and tap water *ad libitum*. The environmental conditions were standardized with respect to temperature, humidity and light. Animal Laboratory Administrative Center and the Institutional Ethics Committee at the National Research Centre, Cairo, Egypt, approved all the experimental procedures.

Acute toxicity

Thirty six (18 male and 18 female) Sprague Dawley rats were divided into 2 main groups namely male and female. Each group was subdivided into 3 groups. Group 1 was received orally a single dose of 1/1024 from the maximum soluble dose of Se (3.8 mg/ kg b.w.). Group 2 was received orally a single dose of the maximum soluble dose of yeast (250 mg/ kg b.w.). Group 3 was received orally a single dose of the maximum soluble dose of Se-Y (250 mg/ kg b.w.). Noteworthy, the Se content of Se-Y was 46.57 µg/ g yeast. The animals were observed for 14 days.

Chronic toxicity

One hundred and eight (54 male and 54 female) Sprague Dawley rats were divided into 2 main groups namely male and female. Each group was subdivided into nine groups. Group 1 served as control group. Groups 2, 3 and 4 and were given three dose levels of Se 3.8, 1.91 or 0.95 mg/kg b. wt. /day respectively for 3 months (These doses represent the high dose, intermediate dose and low dose of Se). Groups 5, 6 and 7 were given three dose levels of yeast 250, 125 and 66.5 mg/kg b. wt. /day respectively for 3 months (These doses represent the high dose, intermediate dose and low dose of yeast). Groups 8, 9 and 10 were given three dose levels of Se-Y 250, 125 and 66.5 mg/kg b. wt. /day

respectively for 3 months (These doses represent the high dose, intermediate dose and low dose of Se-Y).

At the end of the experimental period (3 months), the rats were fasted overnight and subjected to diethyl ether anesthesia. The blood samples were immediately collected from the retro-orbital venous plexus and divided into two portions, the first small portion was taken on EDTA for blood picture analysis, while the second large portion were left to clot in clean dry test tubes, and then centrifuged at 3000 rpm for ten minutes to obtain sera. The clear supernatant sera were then frozen at -20 ºC for the biochemical analyses. After blood collection, the rats were killed and the whole liver and kidney of each rat were rapidly dissected, thoroughly washed with isotonic saline and dried on filter paper, stored in formalin saline (10%) for histopathological investigation.

Hematological analysis

Hemoglobin concentration was determined spectro-photometerically according to the colorimetric method of Drabkin and Austin (1975). White blood cells were counted using improved neubaeur counting chamber, according to the method of Mitruka *et al*. (1977).

Biochemical analyses

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured according to the method of Reitman and Frankel (1957), while serum alkaline phosphatase activity was measured according to the method of Bowers and Mc Comb (1966). Serum urea was assayed according to Henery *et al.*, (1974) and serum creatinin was determined by the method of Murraryy, (1984) using commercial kits.

Histopathological investigation

After fixation of liver and kidney samples of rats in the different groups in 10% formalin saline for twenty four hours, washing was done in tap water. Then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56ºC in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 µm thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain. Then, examination was done through the light electric microscope (Banchroft *et al*, 1996).

Statistical analysis

All data were expressed as the mean ± SE. Statistical analysis was evaluated using the student t-test (Snedecor and Cochran, 1980). P values less than 0.05 were considered as statistically significant.

RESULTS

The present study showed that with the increasing of sodium selenite concentration incorporated into the medium of fermentation, a significant increase (p<0.05) of Se content in the yeast cells was detected. However, with the increase of Se content in the yeast, a significant decrease in dry yeast biomass and cell viability were observed as compared to the blank (**Table 1**).

Table. 1: Influence of incorporation of different concentrations of sodium selenite on cell viability, dry biomass and selenium content of the yeast.

Sodium selenite concentration (µM)	Dead cells (%)	Dry yeast biomass (g)	Selenium content (µg Se/g yeast)
0.0 (blank)	1.0 %	151 ± 1.41	4.60 ± 0.07
19µM	11* %	134 ± 1.30*	46.57 ± 1.58*
39µM	21* %	128 ± 1.14*	138.75 ± 1.14*
57µM	32 *%	117 ± 2.12*	274.57 ± 1.92*

* Significant difference at p<0.05.

Results of Acute Toxicity

The current data revealed that both genders of rats received a single dose of Se (3.8 mg/kg), single dose of yeast (*Saccharomyces cerevisiae*) (250 mg/kg b.wt/ day) or single dose of Se-Y (250 mg/kg) showed no deaths for 14 days. Also, physical observation revealed no changes in the skin, fur, eyes, mucous membranes. Moreover, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma were not detected during 14 days.

Results of Chronic Toxicity

Oral administration of yeast or Se-Y with different doses resulted in no change in blood hemoglobin level in male rats as compared to the control group. In female rats, there was significant reduction (p<0.05) in blood hemoglobin level with oral administration of Se-Y in high and intermediate doses as compared to the control group. Meanwhile, oral administration of Se in the different doses caused significant decrease (p<0.05) in blood hemoglobin level in both male and female rats as compared to the control group. Oral administration of Se, Se-Y or yeast, in all doses except Se-Y in low dose, showed a significant depletion (p<0.05) in white blood cells count in male rats as compared to the control group. Treatment of female rats with Se, Se-Y or yeast in all doses except Se-Y in low dose caused nearly no change in white blood cells count as compared to that in the control group. Low dose of Se-Y supplementation in female rats caused significant increase in white blood cells count compared with that in the control group. Se-Y administration in low dose produced insignificant change (p>0.05) in white blood cells count in male rats (**Table 2**).

Concerning liver enzymes (AST, ALT and ALP) activity, the results revealed a significant increase (p<0.05) in serum AST activity in Se and Se-Y administered groups in high and intermediate doses as compared to the control group in both genders.

Low dose of Se and Se-Y as well as the different doses of yeast produced insignificant change (p>0.05) in serum AST activity in both genders compared with the control group.

Table. 2: Blood hemoglobin level and white blood cell counts among the different studied groups.

Groups	Hemoglobin gm/ dL		WBC $10^3/mm^3$	
	Male	Female	Male	Female
Control	13.50 ± 0.15	13.31 ± 0.16	16.59 ± 0.21	12.57 ± 0.30
High dose Se	11.51 ± 0.13*	11.79 ± 0.36*	13.08 ± 0.56*	10.80 ± 0.85
Intermediate dose Se	11.87 ± 0.55*	11.81 ± 0.43*	13.50 ± 0.21*	12.80 ± 0.74
Low dose Se	12.27 ± 0.46*	11.90 ± 0.31*	15.75 ± 0.19*	13.00 ± 0.77
High dose yeast	14.31 ± 0.61	14.72 ± 0.60	11.80 ± 0.36*	12.13 ± 0.66
Intermediate dose yeast	13.89 ± 0.44	14.69 ± 0.73	12.16 ± 0.38*	12.40 ± 0.49
Low dose yeast	14.84 ± 0.57	14.74 ± 0.26	13.04 ± 0.80*	13.30 ± 0.46
High dose Se-Y	12.40 ± 0.70	10.89 ± 0.28*	12.36 ± 0.20*	12.56 ± 0.84
Intermediate dose Se-Y	12.32 ± 0.69	11.97 ± 0.49*	12.66 ± 0.85*	13.08 ± 0.60
Low dose Se-Y	14.29 ± 0.35	14.41 ± 0.44	15.60 ± 0.87	15.66 ± 0.76*

* Significant difference at $p<0.05$.

Table. 3: serum AST, ALT and ALP activity among the different studied groups.

Group	AST U/L		ALT U/L		ALP U/L	
	Male	Female	Male	Female	Male	Female
Control	56.01±2.10	56.99±1.21	42.57±0.23	43.48±0.36	100.27±1.37	107.38±1.45
High dose Se	92.96±0.65*	87.79±0.97*	75.11±1.09*	70.75±0.65*	153.66±2.01*	156.02±2.25*
Intermediate dose Se	77.03±0.78*	77.61±0.77*	69.02±0.60*	64.12±0.96*	153.36±1.42*	156.21±2.96*
Low dose Se	60.35±0.99	71.82±0.94	53.07±1.27*	55.11±1.80*	141.58±2.35*	149.11±1.43*
High dose yeast	56.63±0.75	57.57±1.60	50.90±0.81	50.94±0.45*	124.48±1.59*	103.92±1.98
Intermediate dose yeast	57.28±1.84	59.58±3.02	47.52±0.93	44.36±1.44	117.32±0.73*	103.91±0.61
Low dose yeast	56.45±2.20	58.42±2.30	44.63±0.37*	44.36±0.211	114.61±1.39*	100.51±0.63*
High dose Se-Y	71.57±2.36*	72.11±0.23*	70.88±1.05*	75.72±0.82*	150.28±1.21*	144.21±1.74*
Intermediate dose Se-Y	69.01±1.92*	71.73±2.30*	58.81±1.25*	64.66±0.65*	144.73±1.35*	137.66±1.59*
Low dose Se-Y	57.63±1.77	59.61±2.44	44.57±0.62*	48.23±0.78*	111.09±0.63*	116.56±1.57*

* Significant difference at $p<0.05$.

Table. 4: Serum urea and creatinin level among the different studied groups.

Groups	Urea nitrogen (mg/dL)		Creatinin (mg/dL)	
	Male	Female	Male	Female
Control	15.28±0.41	16.22±0.40	0.380±0.01	0.491±0.01
High dose Se	34.34±0.87*	31.95±0.79*	0.580±0.03*	0.646±0.03*
Intermediate dose Se	29.67±0.79*	29.87±0.85*	0.570±0.03*	0.575±0.02*
Low dose Se	24.81±0.32*	27.84±0.82*	0.578±0.03*	0.564±0.02*
High dose yeast	18.01±0.38*	19.06±0.17	0.474±0.01*	0.540±0.03
Intermediate dose yeast	15.30±0.58	16.41±0.56	0.437±0.01*	0.548±0.02
Low dose yeast	15.53±0.82	15.15±0.58	0.407±0.01	0.470±0.02
High dose Se-Y	34.71±0.44*	29.16±0.69*	0.542±0.03*	0.621±0.02*
Intermediate dose Se-Y	31.07±0.55*	26.61±0.82*	0.503±0.03*	0.510±0.01
Low dose Se-Y	19.37±0.81*	16.53±0.50	0.415±0.01	0.543±0.04

* Significant difference at $p<0.05$.

Moreover, there was a significant increase ($p<0.05$) in serum ALT activity in Se and Se-Y administered groups of both genders with the different doses as compared to the control group. All doses of yeast in male rats and intermediate as well as low doses of yeast in female rats produced insignificant changes ($p>0.05$) in serum ALT activity as compared to the control group. Administration with Se, yeast or Se-Y in the different doses revealed a significant increase ($p<0.05$) in serum ALP activity in both genders as compared to the control group except the high and intermediate doses of yeast in female rats where they caused insignificant change ($p>0.05$) in serum ALP activity compared with the control group. Moreover, low dose of yeast in female rats exhibited significant decrease ($p<0.05$) in serum ALP activity compared with that in the control group (**Table 3**). The results of kidney function tests showed that administration of Se or Se-Y in the different doses as well as yeast in high dose caused a significant increase ($p<0.05$) in serum urea level in male rats as compared to the control group.

Meanwhile, the intermediate and low doses of yeast in male rats caused insignificant change ($p>0.05$) in serum urea level compared with the control group. Se administration in the different doses and Se-Y in high and intermediate doses caused a significant elevation ($p<0.05$) in serum urea level in female rats as compared to control group. While the different doses of yeast and the low dose of Se-Y in female rats displayed insignificant change ($p<0.05$) in serum urea as compared with that in the control group (**Table 4**).

There was a significant increase ($p<0.05$) in creatinin level among the different groups of male rats treated with Se, yeast or Se-Y except in low dose yeast and Se-Y which showed insignificant change ($p>0.05$) in serum creatinin level compared with the control group. Furthermore, there was a significant increase ($p<0.05$) in creatinin levels in Se in the different doses and Se-Y in high dose in female rats as compared to the control group. Different doses of yeast and the intermediate as well as low dose of Se-Y in female rats caused insignificant change ($p>0.05$)

in serum creatinin level as compared with that in the control group (**Table 4**).

Histopathological investigation of liver in the different studied groups

Microscopic examination of liver tissue section of control male and female rats showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein (**Figs.1A and 1B**).

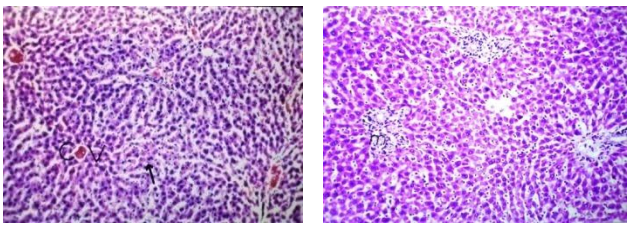

Fig. 1: Photomicrographs of liver tissue section of control normal group. (**A and B**) normal male and female rats showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein.

Supplementation with high dose Se as sodium selenite (3.8 mg/kg) for 3 months in both male and female rats showed edema with inflammatory cells infiltration in the portal area in both male and female rats (**Figs. 2A and 2B**).

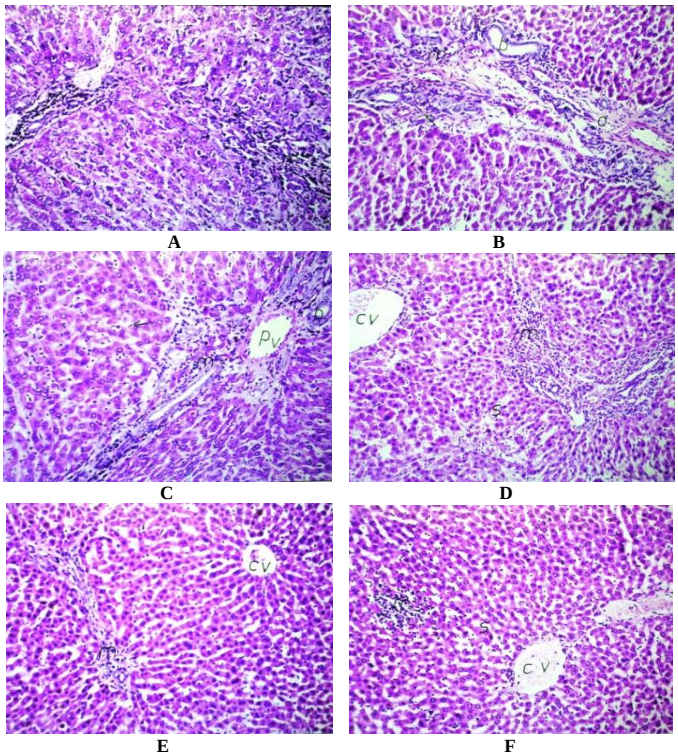

Fig. 2: Photomicrographs of liver tissue sections of both male and female rats administered with different doses of Se. (**A and B**) male and female rats administered with high dose of Se, showed edema with inflammatory cells infiltration in the portal area. (**C and D**) male and female rats administered with intermediate dose of Se showed inflammatory cells infiltration in the portal area associated with dilatation in the portal vein and sinusoid. (**E and F**) male and female rats administered with low dose of Se showed inflammatory cells infiltration in the hepatic parenchyma in male rats (**Fig. 2E**) associated with dilatation in the central vein and sinusoid in female rat (**Fig 2F**).

Examination of liver sections of both male and female rats administered with intermediate dose of Se (1.91mg/kg sodium selenite) for 3 months showed inflammatory cells infiltration in the portal area associated with dilatation in the portal vein and sinusoid in both male and female rats (**Figs.2C and 2D**). Following oral administration of low dose Se (0.98 mg/kg sodium selenite) for 3 months, inflammatory cells infiltration were detected in the hepatic parenchyma in male rats (**Fig.2E**) associated with dilatation in the central vein and sinusoid in female rat (**Fig.2F**). Supplementation with high dose of yeast (*Saccharomyces cerevisiae*) (250 mg/kg) for 3 months in both male and female rats showed that there was no histopathological alteration in the liver of male rats (**Fig.3A**), while there was few inflammatory cells infiltration in the portal area in female rats (**Fig. 3B**). Examination of liver sections of both male and female rats administered with intermediate dose from *S. cerevisiae* (125 mg/kg) for 3 months showed that there was no histopathological alteration in male rats (**Fig. 3C**). However, inflammatory cells infiltration were detected in the portal area as well as in between the hepatocytes in female rats (**Fig. 3D**). Following the administration of low dose of *S.cerevisiae* (66.5 mg/kg) for 3 months, there was no histopathological alteration in the liver sections of both male and female rats (**Figs. 3E and 3F**).

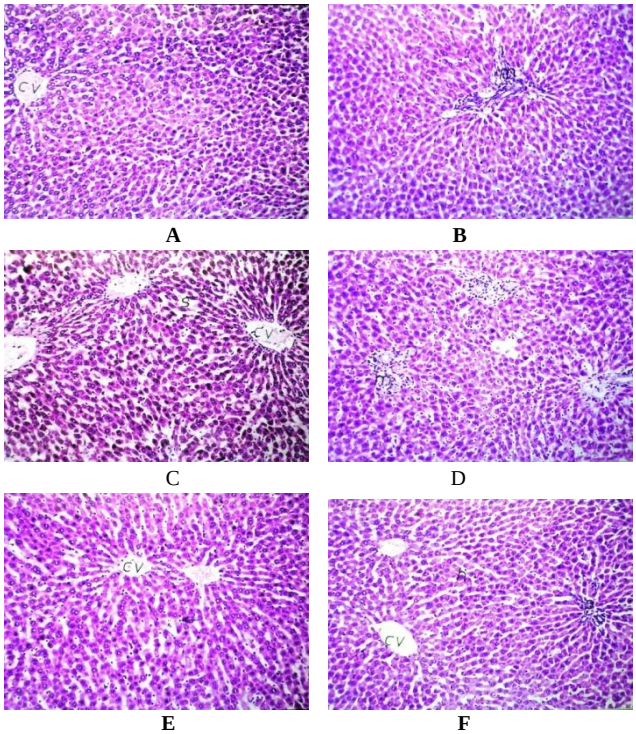

Fig. 3: Photomicrographs of liver tissue sections of both male and female rats administered with yeast (*S. cerevisiae*). **(A and B)** male and female rats administered with high dose *S. cerevisiae* showed that there were no histopathological observed in male rats **(Fig. 3A)** and few inflammatory cells infiltration in portal area in female rats **(Fig 3B). (C and D)** male and female rats administered with intermediate dose *S. cerevisiae*, showed that there was no histopathological alteration in male rats **(Fig. 3C).** Inflammatory cells infiltration were observed in the portal area as well as in between the hepatocytes in female rats **(Fig. 3D). (E and F)** male and female rats administered with low dose *S. cerevisiae* showed that there was no histopathological alteration in both male and female rats.

Supplementation with high dose Se-Y (250 mg/kg) for 3 months in both male and female rats showed few inflammatory cells infiltration in the portal area of liver in male rats (**Fig. 4A**) with diffuse kupffer cells proliferation in between the hepatocyte in female rats (**Fig. 4B**). Examination of liver sections of both male and female rats administered with intermediate dose from Se-Y (125 mg/kg) for 3 months showed dilatation in the portal vein and sinusoid in association with edema in the portal area in male rats (**Fig. 4C**), and there was no histopathological alteration in liver tissue of female rats (**Fig. 4D**).

Following the administration of low dose of Se-Y (66.5 mg/kg) for 3 months, there was no histopathological alteration in the liver tissue of both male and female rats (**Figs. 4E and 4F**).

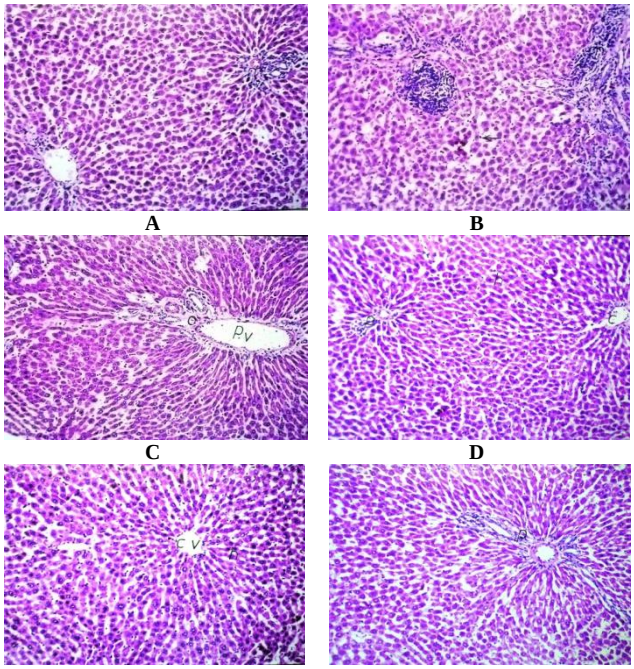

Fig. 4: Photomicrographs of liver tissue sections of both male and female rats administered with Se-Y. (A and B) male and female rats administered with high dose Se-Y showed few inflammatory cells infiltration in the portal area in male rats (fig. 4A) with diffuse kupffer cells proliferation in between the hepatocyte in female rats (Fig. 4B). (C and D) male and female rats administered with intermediate dose Se-Y showed dilatation in the portal vein and sinusoid in association with edema in the portal area in male rats (Fig 4C), and there was no histopathological alteration in female rats (Fig 4D). (E and F) male and female rats administered with low dose Se-Y showed that there was no histopathological alteration observed in both male and female rats.

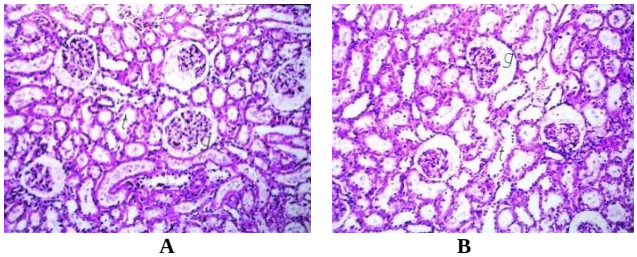

Fig. 5: Photomicrographs of kidney tissue section of control group. (**A and B**) normal male and female rats showed normal appearance of glomeruli, bowman capsule, proximal and distal tubules.

Histopathological investigation of kidney in the different studied groups

Microscopic examination of kidney tissue section of control male and female rats showed normal appearance of glomeruli, bowman capsule, proximal and distal tubules (**Figs.5A and 5B**).

Supplementation with high dose Se as sodium selenite (3.8 mg/kg) for 3 months in both male and female rats showed focal inflammatory cells aggregation surrounding the blood vessels at the cortex of the kidney in both male and female rats (**Figs.6A and 6B**). Examination of kidney tissue sections of both male and female rats administered with intermediate dose from Se (1.91mg/kg sodium selenite) for 3 months showed sever dilatation and congestion in the blood vessels associated with degeneration in the lining epithelium of the tubules at the cortex in both male and female rats (**Fig. 6C and 6D**). Following oral administration of low dose of Se (0.98 mg/kg sodium selenite) for 3 months, there was dilatation in the blood vessels associated with few inflammatory cells infiltration in the perivascular tissue at the cortex in male rats (**Fig.6E**) and there was degenerative change in the lining epithelium of the renal tubules in female rats (**Fig. 6F**).

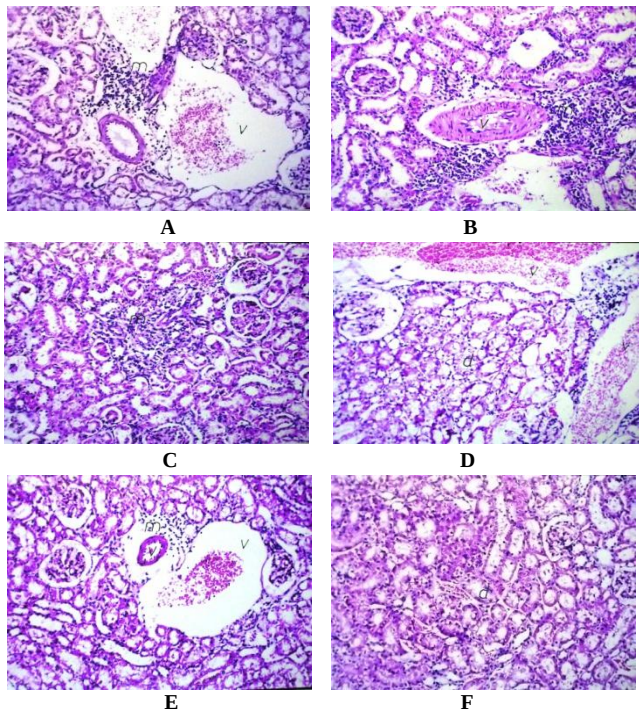

Fig. 6: Photomicrographs of kidney sections of both male and female rats supplemented with different doses of Se. (**A and B**) male and female rats administered with high dose of sodium selenite, showed focal inflammatory cells aggregation surrounding the blood vessels at the cortex of the kidney in both male and female rats. (**C and D**) male and female rats administered with intermediate dose of sodium selenite, showed sever dilatation and congestion in the blood vessels associated with degeneration in the lining epithelium of the tubules at the cortex of the kidney in both male and female rats. (**6E and 6F**) male and female rats administered with low dose of sodium selenite, there was dilatation in the blood vessels associated with few inflammatory cells infiltration in the perivascular tissue at the cortex in male rats (**Fig.6E**), and there was degenerative change in the lining epithelium of the renal tubules in female rat (**Fig. 6F**).

Supplementation with high dose yeast (*Saccharomyces cerevisiae*) (250 mg/kg) for 3 months in both male and female rats showed focal inflammatory cells infiltration in between the tubules and glomeruli in male rats (**Fig. 7A**). Congestion was detected in the cortical blood vessels in female rats (**Fig. 7B**). Examination of kidney tissue sections of both male and female rats administered with intermediate dose from *S. cerevisiae* (125 mg/kg) for 3 months showed focal inflammatory cells infiltration in between the degenerated tubules in the cortex of both male and female rats (**fig 7C and 7D**). Following oral administration of low dose of *S.cerevisiae* (66.5 mg/kg) for 3 months, there was no histopathological alteration in kidney tissue sections of both male and female rats (**Figs. 7E and 7F**).

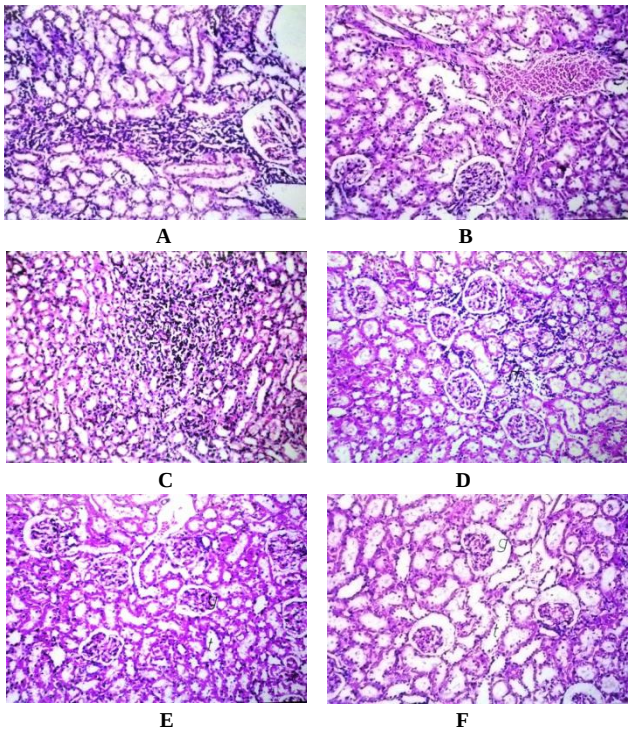

Fig.7. Photomicrographs of kidney sections of both male and female rats administered with different doses of yeast (*S. cerevisiae*). (**A and B**) male and female rats administered with high dose *S. cerevisiae* showed focal inflammatory cells infiltration in between the tubules and glomeruli in male rats (**Fig. 7A**). Congestion was detected in the cortical blood vessels in female rats (**Fig 7B**). (**C and D**) male and female rats administered with intermediate dose *S. cerevisiae* showed focal inflammatory cells infiltration in between the degenerated tubules in the cortical portion in both male and female rats. (**E and F**) male and female rats administered with low dose of *S. cerevisiae* showed that there was no histopathological alteration in both male and female rats.

Supplementation with high dose of Se-Y (250 mg/kg) for 3 months in both male and female rats showed dilatation and congestion in the blood vessels at the cortex of the kidney in both male and female rats (**Figs. 8A and 8B**).

Examination of kidney tissue sections of both male and female rats administered with intermediate dose of Se-Y (125 mg/kg) for 3 months showed focal inflammatory cells infiltration in between the tubules of male rats (**Fig. 8C**) and there was degenerative change in the tubular lining epithelium of female rats (**Fig. 8D**). Following administration of low dose Se-Y (66.5 mg/kg) for 3 months, there was no histopathological alteration in the kidney tissue sections of both male and female rats (**Figs. 8E and 8F**).

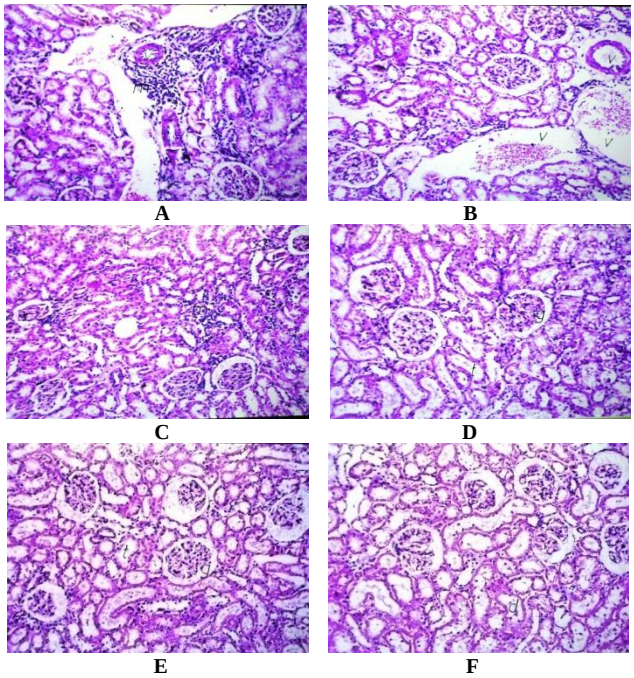

Fig.8. Photomicrographs of kidney sections of both male and female rats administered with Se-Y. (**A and B**) male and female rats administered with high dose Se-Y, showed dilatation and congestion in the blood vessels at the cortex of the kidney in both male and female rats. (**C and D**) male and female rats administered with intermediate dose of Se-Y showed focal inflammatory cells infiltration in between the tubules in kidney of male rats (**Fig. 8C**) and degenerative change in the tubular lining epithelium in kidney of female rats (**Fig. 8D**). (**E and F**) male and female rats administered with low dose Se-Y showed that there was no histopathological alteration in both male and female rats.

DISCUSION

The obtained results revealed that increased Se concentration incorporated into the medium of fermentation, produced significant increase in Se content in the yeast cells accompanied by significant decrease in each of dry yeast biomass and cell viability. Results of experiments on selenium accumulation in *S. cerevisiae* indicate that the optimization of selenium concentration in the nutrient medium is a necessity (Suhajda *et al*., 2000). The decrease in cell growth due to Se concentration could be attributed to selenium's role as a toxic and growth inhibiting element by formation of selenium binding proteins (SBP) and/or by modulating properties of growth regulatory protein. Using excess amounts of Se compounds has dramatic effects upon the viability of the cells, cell cycle, protein synthesis and DNA integrity when studied in cell culture (Spallholz, 1994). Selenite has been found to change glutathione, GSH: GSSG ratio *via* GSH oxidation thus, inhibiting the G1, G2 and S-phases of cell division and protein synthesis, in addition, selenite can cause DNA damage and induce apoptosis. (Jr.Combs and Grey, 1998). Anti-oxidative defenses working against oxidative stress can however improve the life span of yeast cells.

Studies by Wawryn *et al*. (1999) have shown that deficiencies in the antioxidant enzymes result in an almost threefold shortening of the life span of individual yeast cells. Thus, proper enhancement of the anti-oxidative defense status seems to be beneficial for the growth and survival of yeast cells.

In the present study, we achieved yeast enriched with Se by supplementing yeast with inorganic form of sodium selenite in appropriate concentration and getting a balance between Se incorporation and optimum growth of the yeast cells along with yeast enhanced anti-oxidative defense status. During the growth of *S. cerevisiae* yeast, selenite, which is a potentially toxic and poorly bioavailable species, is converted enriched into a safer and highly bioactive species with improved nutritional properties. Se-Y has been thus viewed as a safe and effective form of Se supplementation (Kaur and Bansal, 2006).

The present study showed that administration of Se in the different doses caused a decrease in blood Hb level. Se supplementation in an inappropriate dose could affect protein synthesis in addition to its ability to bind with protein to form Se binding proteins (Spallholz, 1994). Thus, the reduction of blood Hb level in Se supplemented rats could be attributed to the toxic effect of this element. In accordance with our results, it has been found that individuals live in high Se soil areas showed some pathological symptoms including nausea, vomiting, skin depigmentation, hair loses and low blood hemoglobin level (Burke, 1976).

In the present study, Se administration in high or intermediate dose caused a decrease in WBC counts while low dose of Se caused nearly no change in WBC count. These results are in agreement with Hawkes *et al.* (2001) who reported that WBC count decreased in the high Se group and increased in the low Se group, as a result of the changes in granulocytes. The biological importance of Se is at least 3-folds: First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine 5-deiodinase, and thioredoxin reductase (Stadtman, 1996). Second, sodium selenite is protective against a number of toxicants. Third, selenium excessive intake causes toxic potential (Jr. Combs and Gray, 1998).

In the view of the obtained data, oral administration of Se in high or intermediate dose caused elevation in ALT, AST and ALP activity while low dose of Se caused nearly no change in the activity of ALT, AST and ALP. The biochemical mechanism for the toxicity of Se and related compound is the mediation of oxidative stress mechanisms (Quadrani *et al.*, 2000). It has been found that excess Se could inhibit dehydrogenase enzymes, and remove the sulfhydryl groups essential to cellular oxidative processes (Watts, 1994). Thus, excess Se could produce hepatocellular injury. AST and ALT are two enzymes of the most reliable markers of hepatocellular injury. Their values are elevated in serum of patients with a variety of hepatic disorders. Of the two, ALT is thought to be more specific for hepatic injury because it is present mainly in liver cytosole and in low concentration elsewhere (Giboney, 2005). When the liver hepatocytes are damaged, these enzymes are released into the blood where the significant increase in AST and ALT activities indicates the damage to the cytosole and also to mitochondria (Mathuria and Verma, 2008). Therefore, it could be suggested that the oxidative stress may mediate the disturbance in hepatic function due to excess Se which is reflected by the present increase in serum ALT and AST activities.

Our results indicated that administration of the different doses of Se increase serum urea and creatinin levels as compared to the control group. Se mediates apoptotic and free radical formation (Hunsaker *et al.*, 2005). Impairment in kidney function could probably occur as a result of kidney oxidative damage (Mahjoubi-Sam *et al.*, 2008). Urea and creatinin are waste products of protein metabolism that need to be excreted by the kidney. Therefore marked increase in serum urea and creatinin levels confirms the functional damage of the kidney (Garba *et al.*, 2007). Creatinin level is more specific to the kidney function, since kidney damage is the only significant factor that increases serum creatinin level (Nwanjo *et al.*, 2005).

The present results showed that yeast administration in the different doses resulted in nearly no change in the studied biochemical parameters. The polysaccharide beta-glucane, one of the major cell wall components of *S. cerevisiae*, acts as nonspecific immune system stimulant (Cross *et al.*, 2001; Kogan *et al.*, 2002). Krizkova *et al.* (2000) stated that *S. cerevisiae* cell wall mannan has relatively good antioxidative effects as it is able to scavenge reactive oxygen radicals. Masson and Ramotar (1996) proposed that *S. cerevisiae* c-IMP2 gene prevents the oxidative damage by regulating the expression of genes that are directly required to repair DNA damage. The obtained results revealed that, there was no changes in ALT, AST and ALP activities in the groups that received yeast (*S. cerevisiae*). These results are in agreement with Bernard *et al.* (2000) who reported that, the dietary supplementation with yeast derived beta-glucan and single-strain probiotics did not have significant effects on the activities of AST and ALT in broiler chicks. Also, Al-Kassie *et al.* (2008) reported that no effects on serum ALT and AST activities were observed by the addition of probiotic (*Saccharomyces cerevisiae*) rich in beta-glucan compared with the control. Also, our results are in agreement with Mannaa *et al.* (2005) who reported that there is no marked adverse action of the yeast on rat liver with an intangible increase in serum ALT, ALP activities.

The obtained data revealed that administration of Se-Y in high or intermediate dose caused significant increase in ALT, ASL, ALP activities, urea and creatinin levels as compared to the control group. These findings could be attributed to the hifg Se content in these doses of Se-Y. However, the chronic toxicity study on Se-Y has been reported to be lower compared to sodium selenite in experiments with weanling rats (Spallholz and Rafferty, 1987). Meanwhile, administration of Se-Y in low dose resulted in nearly no changes in these biochemical parameters. The obtained results are in agreement with Yoshida *et al.* (1999) who reported that Se in Se-Y is more biovailable than selenite, and therefore it is the preferred form for Se supplementation. Close similarity of

selenized yeast to the natural forms found in feed crops, plus the careful control of Se content that can be exercised in yeast production, makes Se-yeast to be a most interesting, useful and environmentally-safe supplementary material for use with livestock. Histopathological investigation showed that supplementation with Se in the different doses caused edema and inflammatory cell infiltration in the portal area associated with dilation in centrals and sinusoids. Also, there was degenerative change in the lining epithelium of the renal tubules, dilation in the blood vessels associated with few inflammatory cells infiltration in the perivascular tissue at the cortex. These findings agree with the previous report of Manikandon *et al.* (2010) who reported that Se administration causes liver to be infiltrated with mononuclear cells, vaculation and necrosis as well as it pronounce degeneration. And kidney from Se alone administered rats showed vacuolar degeneration changes in the epithelial cells, cellular proliferation with fibrosis, thickening of capillary walls and glomerular tuft atrophy.

Administration of yeast (*S. cerevisiae*) in high and intermediate doses causes inflammatory cell infiltration in the portal area of the liver. There was no histopathological alteration observed in low dose of yeast. These findings are in accordance with those of Mannaa *et al.* (2005) who reported that in yeast-treated animals, regular arrangement of hepatic cords is clearly observed. The sinusoids are of normal arrangement and size and binucleated hepatocytes are abundant. Also, our results revealed that there was no histological alteration in kidney tissue sections in low dose of *S.cerevisiae*, theses findings are in agreement with Darwish *et al.* (2011), who reported that the administration of *S.cerevisiae* showed normal architecture of rat kidney.

Administration of Se-Y in high or intermediate dose caused inflammatory cells infiltration associated with edema in the portal area of the liver. Also, there was dilation and congestion in the blood vessels at the cortex of the kidney. However, there was no histological alteration in low dose of Se-Y in both liver and kidney tissue. These findings are in agreement with Mirjana *et al.* (2004) who reported that no alteration were noticed in the liver and kidney supplied with low dose Se in the form of selenized yeast. Alterations of liver and kidney were encountered only with exceptionally high levels of organic selenium.

In conclusion, the current study revealed that 19 μM sodium selenite could be the proper concentration for achieving optimal growth of yeast cells. Also, our study declared the safety of chronic administration of Se enriched yeast compared with inorganic Se in the form of sodium selenite.

ACKNOWLEDGMENTS

The authors express sincere appreciation to prof. Adel Bakeer Kholoussy, Professor of pathology, Faculty of Veterinary Medicine, Cairo University for his kind cooperation in conducting histopathological investigations in this study. The authors acknowledge the financial support of the National Research Centre, Egypt (grant no: 9100305- 2011-2013).

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How to cite this article:

Hanaa H. Ahmed, EL-S M.E. Mahdy, Hatem A. EL-mezayen, Rehab M. Hussien. Assessment of the Safety of Chronic Administration of Selenium Enriched Yeast. J App Pharm Sci, 2013; 3 (06): 136-145.