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Research Article

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Evidences for the antifibrotic activity of selenium-enriched yeast in *in vivo* model of liver fibrosis

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ABSTRACT

This study aimed at evaluating the protective role of Se-Y against TAA induced hepatotoxicity. Sixty-four adult female rats were divided into 8 group: (1) served as control, (2) was administered TAA, (3 and 4) were given two dose levels of Se (0.95 and 0.47 mg kg^{-1} day^{-1}) respectively with simultaneous administration of TAA, (5 and 6) were given two dose levels of yeast (66.5 and 33.25mg kg^{-1} day^{-1}) respectively with simultaneous administration of TAA and (7 and 8) were given two dose levels of Se-Y (66.5 and 33.25mg kg^{-1} day^{-1}) respectively with simultaneous administration of TAA. Serum ALT, AST, ALP activities, TNF-α, MCP-1, IL-6, IL-8, laminin and fibronectin levels were determined. Histopathological examination of liver tissue sections was carried out. The results revealed that TAA intoxication caused significant increase in serum ALT, AST, ALP activities, TNF-α, MCP-1, IL-6, IL-8, laminin and fibronectin levels versus the control. Histopathological investigation of liver tissue sections of TAA intoxicated rats showed necrosis with inflammatory cell infiltration. Co-treatment with Se, yeast or Se-Y produced significant protection against TAA-induced hepatotoxicity as indicated by marked improvement in the studied biochemical markers. In conclusion, Se, yeast and Se-Y could protect the rat liver from TAA induced hepatotoxicity.

Key words: hepatotoxicity, selenium, yeast, thioacetamide

INTRODUCTION

Liver is one of the vital organs of the animal body and plays a central role in transforming and clearing the chemicals, but is susceptible to the toxicity from these agents. Some chemical agents, such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxins. More than 900 drugs have been implicated in causing liver injury and its one of the most common reasons for a drug to be withdrawn from the market [1]. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. About 75%-80% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen *via* portal veins which bring drugs and xenobiotics in concentrated form [2]. Injury to hepatocyte and bile duct cells leads to accumulation of bile acid inside the liver, which promotes further liver damage [3].

Thioacetamide (TAA) is employed as a curing ingredient, a chemical reagent, a raw medicine, a pesticide, a textile dye and a finishing auxiliary [4]. TAA, a potent hepatotoxicant, under-goes a two-step bioactivation mediated by cytochrome P450 to TAA sulfoxide (TASO), and further to TA-S,S- dioxide ($TASO_2$), a reqactive metabolite that initiates cellular necrosis [5].

Selenium (Se) is one of the most important microelements necessary for correcting the vital functions of living organisms. In oral preparations, Se occurs in the form of sodium selenite and methionine selenide, whose physiological activity is well documented [6]. The biological importance of Se is at least 3-fold. First, it forms the prosthetic group of some critical selenocysteine-containing enzymes, such as glutathione peroxidase, iodothyronine

5,,-deiodinase, and thioredoxin reductase [7]. Second, sodium selenite is protective against a number of toxicants, Third, Se excessive intake cause toxic potential [8].

Hartwell *et al.* [9] have pioneered a noval approche in which the yeast *Saccharomyces cerevisiae* is used to discover compounds with powerful chemotherapeutic potential. The yeast *Saccharomyces cerevisiae* contains a number of the essential antioxidant trace elements mainly zinc in addition to cobalt, together with yeast cell wall mannans [10], the poly saccharide beta-glucan [11] and the IMP2 gene [12]. In addition, it contains the antioxidative cytosolic enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) together with mitochondrial Mn-SOD, Sodium selenite and vitamins A, B_{12} and E [13]. Mannaa *et al.* [14] demonstrated that each one of the components in *Saccharomyces cerevisiae* effectively participates in attenuation of the oxidative stress caused by chemotherapy and in promoting regeneration of new hepatocytes and mean while restoring liver function beyond normal status.

The 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, a most interesting, useful and environmentally-safe supplementary material for use with livestock.

This study was designed to assess the hepatoprotective activity of selenium-enriched yeast (Se-Y) against thioacetamide-induced hepatotoxicity in rats and to prove the scientific base of traditional use of Se-Y against liver disorder.

EXPERIMENTAL SECTION

Materials

Yeast strain, *Saccharomyces 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). YEPD medium used in the present study contained the following components: 3g Yeast extract, 10 g peptone, 20 g dextrose in one liter of distilled water with final pH4.5. The yeast strain was maintained on the YEPD agar slants **(**2.5% agar).Yeast, *Saccharomyces 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 [15].

Preparation of selenium- enriched-yeast

Se in inorganic form as sodium selenite **(**Na2SeO3) was used. From 25 mM sodium selenite stock solution, 19 µM Se concentration was made in different 20 ml aliquots of yeast culture media. Based on our previous work [16], 19 µM of Se concentration in yeast is the proper concentration for achieving optimal growth of yeast cells. These media with Se concentration was inoculated with 100 µl of overnight culture and incubated at 30°C for overnight growth on shaker [15].

Animals

Sixty-four adult female rats weighing 150-170 g were obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were acclimatized for one week in a specific pathogen free (SPF) barrier area where the temperature was 25±1 and humidity was 55%. Rats were controlled constantly with a 12 h light/dark cycle at National Research Centre Animal Facility Breeding Colony. Rats were housed with *adlibitum* access standard laboratory diet consisting of casein 10%, salts mixture 4%,vitaminsmixture 1%,corn oil 10%and cellulose 5%completed to 100 g with corn starch [17]. Animal cared for according to the guidelines for animal experiments which were approved by the Ethical Committee of Medical Research of the National Research Centre, Cairo, Egypt.

Experimental design

The animals were divided into 8 groups (8 rats/ group).Group1 served as normal control group. Group 2 was administered TAA (100 mg kg^{-1} b.w day^{-1}) [18] for 6 weeks. Groups 3 and 4, were administered Se (0.96 or 0.48 mg kg^{-1} b.w day^{-1} respectively), simultaneously with TAA for 6 weeks. Groups 5 and 6 were administered yeast (66.5 or 33.25 mg kg^{-1} b.w day^{-1} respectively), simultaneously with TAA for 6weeks. Groups 7 and 8 were administered with Se-Y (66.5 or 33.25 mg kg^{-1} b.w day^{-1} respectively), simultaneously with TAA for 6 weeks. The selected doses of each of Se, yeast and Se-Y were chosen according to the chronic toxicity study done in our previous work [16].

At the end of the experimental period, the rats were fasted overnight and subjected to diethyl ether anesthesia. The blood samples were immediately withdrawn from the retro orbital venous plexus in clean tubes and then centrifuged at 1800xg at 4 °C for 15 min to separate sera.

After blood collection, the liver of each rat was rapidly dissected, washed in isotonic saline, and stored in formalin saline (10%) for histopathological investigation.

Methods

Biochemical analyses

Serum aspartate transaminase **(**AST) and alanine transaminase **(**ALT) activities were measured according to the method of Reitman and Frankel, [19] and serum alkaline phosphatase activity (ALP) was estimated according to the method of Bowers and McComb, [20]. Serum TNF-α level was carried out using TNF-α ELISA assay kit purchased from Koma Biotech Co., Korea according to the instruction provided. Serum monocyte chemotactic protein (MCP-1), IL-6, IL-8 and laminin levels were assayed using ELISA assay kits purchased from Glory Science Co., New York (U.S.A) according to the instruction provided. Serum fibronectin level was carried out using fibronectin ELISA kit according to the method of Hynes, [21].

Histopathological investigation

After fixation of liver sample of each rat in different studied 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 beeswax tissue blocks were prepared for sectioning at 4µm thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin stain. Then, examination was done through the light electric microscope [22].

Statistical analysis

All data were expressed as mean ± SE. Statistical analysis was done using the student t-test [23]. *P* values less than 0.05were considered statistically significant. Percentage difference representing the percent of variation with respect to the corresponding control group was calculated using the following formula

$$\% \text{ difference} = \frac{\text{treated value} - \text{Control value}}{\text{Control value}} \times 100$$

RESULTS AND DISCUSSION

In the current work, the administration of TAA produced serious attack on liver as indicated by the significant increase ($p<0.05$) in each of AST, ALT and ALP activities as compared to the normal control group. The increment of AST activity was 131.075 and that for ALT activity was 194.90% as well as that for ALP activity was 76.40% versus their corresponding values in the normal control (Table 1).The elevated serum liver enzymes (AST and ALT) activity is an indicator of cellular liver necrosis [24]. TAA is a well-established tool to induce hepatotoxicity specially necrosis in experimental animal models [25]. The elevated serum AST and ALT activities in the TAA intoxicated group could be attributed to the leakage of the enzymes from liver cells as a result of hepatic tissue damage [26]. TAA is able to produce different grades of hepatic damage, including centrilobular necrosis [27]. Moreover, in our results, TAA intoxication resulted in a significant increase ($p<0.05$) in serum ALP activity, this result is in agreement with Giffen *et al.* [28]. This finding could be attributed to the generation of free radicals after TAA intoxication which could affect the hepatic cellular permeability leading to elevation in circulating level of this enzyme [29].

The data shown in table 2 revealed a significant increase ($p<0.05$) of serum TNF-α, MCP-1, IL-6 and IL-8 levels in TAA-intoxicated group as compared to the control group. The percentage of increment was 33.12% for TNF-α, 44.56% for MCP-1, 65.79% for IL-6 and 70.28% for IL-8 levels versus their corresponding values in the normal control. During the development of hepatic inflammation, the hepatocytes are exposed to the increased levels of cytokines such as TNF-α, MCP-1, IL-6 and IL-8 [30]. These cytokines are regulated by nuclear factor kappa-B (NF-κB) [31]. Oxidative stress which is induced by excess reactive oxygen species (ROS) could activate NF-κB [32]. Furthermore, as a transcription factor, NF-κB regulates expression of many kinds of cytokines in the liver. Once NF-κB is activated, the gene expression of TNF-α, MCP-1 and IL-6 is inordinately increased [33]. IL-8 secretion is complex and is regulated primarily at the transcriptional level through cooperative interactions of the transcription factors NF-κB and IL-6 in tissue [34].

Table (1): Effect of Se, yeast "*Saccharomyces cerevisiae*" and Se-Y on serum parameters of liver functions of TAA-intoxicated female rats

	AST (U/L)	ALT(U/L)	ALP(U/L)
Control	56.99 ± 0.47	43.48 ± 0.85	107.38± 0.58
TAA	131.69 ± 1.45$^{\blacklozenge *}$ (131.07%)	128.24 ± 1.93$^{\blacklozenge *}$ (194.90%)	189.42 ± 0.90$^{\blacklozenge *}$ (76.40%)
High dose Se	88.18 ± 1.05$^{\cdot *}$ (-33.03%)	70.97 ± 1.67$^{\cdot *}$ (-44.65%)	149.27 ± 1.78$^{\cdot *}$ (21.19%)
Low dose Se	95.76 ± 0.96$^{\cdot *}$ (-27.28%)	85.17 ± 2.01$^{\cdot *}$ (-33.58%)	163.46± 2.77$^{\cdot *}$ (13.70%)
High dose yeast	92.42 ± 2.72$^{\cdot *}$ (-29.82%)	76.78 ± 0.95$^{\cdot *}$ (-40.12%)	152.18± 1.14$^{\cdot *}$ (19.66%)
Low dose yeast	104.16 ± 2.26$^{\cdot *}$ (-20.90%)	87.76 ± 0.75$^{\cdot *}$ (-31.56%)	168.37± 2.34$^{\cdot *}$ (11.00%)
High dose Se-Y	76.56 ± 0.78$^{\cdot *}$ (-41.86%)	58.95 ± 1.63$^{\cdot *}$ (-54.03%)	137.57± 1.59$^{\cdot *}$ (27.37%)
Low dose Se-Y	99.34 ± 1.30$^{\cdot *}$ (-24.56%)	71.05 ± 0.39$^{\cdot *}$ (-44.59%)	156.40± 1.46$^{\cdot *}$ (17.43%)

$^{\blacklozenge}$ *Difference in relation to control group.* * *Significant change at p< 0.05.*
$^{\cdot}$ *Difference in relation to TAA-intoxicated group.*

Table (2): Effect of Se, yeast "*Saccharomyces cerevisiae*" and Se-Y on *serum TNF-α, MCP-1, IL-6 and IL-8 in TAA-intoxicated rats*

	TNF-α (pg/ml)	MCP-1 (ng/L)	IL-6 (pg/ml)	IL-8 (ng/L)
Normal control	79.48 ± 1.0	65.08 ± 2.3	343.56 ± 3.32	4.61±0.43
TAA (100 mg kg^{-1} b.w/day)	105.81 ±2.1$^{\blacklozenge *}$ (33.12%)	94.08 ±2.7$^{\blacklozenge *}$ (44.56%)	569.6 ±5.17$^{\blacklozenge *}$ (65.79%)	7.85±1.52$^{\blacklozenge *}$ (70.28%)
Se (0.96 mg kg^{-1} b.w/day)	87.69 ± 1.4$^{\cdot *}$ (-17.12%)	81.93 ±2.9$^{\cdot *}$ (-12.91%)	415.8 ±6.85$^{\cdot *}$ (-26.99%)	6.08±0.17$^{N.S}$ (-22.54%)
Se (0.48 mg kg^{-1} b.w/day)	95.07 ±0.83$^{\cdot *}$ (-10.15%)	86.00 ±2.7$^{\cdot *}$ (-8.58%)	474.8 ±7.03$^{\cdot *}$ (-16.63%)	6.66±0.23$^{N.S}$ (-15.15%)
Yeast (66.5 mg kg^{-1} b.w/day)	87.38 ±0.50$^{\cdot *}$ (-17.41%)	82.52 ±1.7$^{\cdot *}$ (-12.28%)	444.28 ±4.7$^{\cdot *}$ (-22.00)	6.11±0.29$^{N.S}$ (-22.16%)
Yeast (33.25 mg kg^{-1} b.w/day)	99.79 ±3.95$^{\cdot *}$ (-5.6%)	84.40 ±2.1$^{\cdot *}$ (-10.28%)	488.1 ±4.27$^{\cdot *}$ (-14.31%)	6.76±0.21$^{N.S}$ (-13.18%)
Se-Y (66.5 mg kg^{-1} b.w/day)	84.22 ± 1.3$^{\cdot *}$ (-20.40%)	75.69 ±1.6$^{\cdot *}$ (-19.54%)	410.4 ±4.82$^{\cdot *}$ (-27.95%)	5.10±0.14$^{\cdot *}$ (-35.03%)
Se-Y (33.25 mg kg^{-1}b.w/day)	99.70 ±1.84$^{\cdot *}$ (-5.20%)	82.14±2.5$^{\cdot *}$ (-12.69%)	450.96 ±7.3$^{\cdot *}$ (-20.82%)	5.68±0.20$^{\cdot *}$ (-27.64%)

$^{\blacklozenge}$ *Difference in relation to control group.* * *Significant change at p< 0.05.*
$^{\cdot}$ *Difference in relation to TAA-intoxicated group.*

Table (3): Effect of Se, yeast "*Saccharomyces cerevisiae*" and Se-Y on serum laminin and fibronectin in TAA-intoxicated rats

	Laminin (µg/L)	Fibronectin (ng/ml)
Normal control	57.90 ± 0.48	922.00 ± 14.00
TAA (100 mg/ kg b.w/ day)	136.79 ± 1.75$^{\blacklozenge *}$ (136.25%)	1603.66 ± 28.67$^{\blacklozenge *}$ (73.93%)
Se (0.96 mg/ kg b.w/ day)	80.69 ± 2.18$^{\cdot *}$ (-41.01%)	1361.00 ± 48.18$^{\cdot *}$ (-15.13%)
Se (0.48 mg/ kg b.w/ day)	87.90 ± 2.56$^{\cdot *}$ (-35.74%)	1435.00 ± 33.10$^{\cdot *}$ (-10.48%)
Yeast (66.5 mg/ kg b.w/ day)	83.65 ± 2.60$^{\cdot *}$ (-38.84%)	1358.16 ± 35.92$^{\cdot *}$ (-15.30%)
Yeas (33.25 mg/ kg b.w/ day)	92.98 ± 0.73$^{\cdot *}$ (-32.02%)	1412.83 ± 22.08$^{\cdot *}$ (-11.89%)
Se-Y (66.5 mg/ kg b.w/ day)	70.63 ± 1.58$^{\cdot *}$ (-48.36%)	1132.83 ± 16.17$^{\cdot *}$ (-29.35%)
Se-Y (33.25 mg/ kg b.w/ day)	78.09 ± 2.65$^{\cdot *}$ (-42.91%)	1217.50 ± 28.68$^{\cdot *}$ (-24.07%)

$^{\blacklozenge}$ *Difference in relation to control group.* * *Significant change at p< 0.05.*
$^{\cdot}$ *Difference in relation to TAA-intoxicated group.*

The present findings revealed significant increase (p<0.05) in serum laminin and fibronectin levels in TAA-intoxicated group as compared to the control group (Table 3). The percentage of increment was 136.25% for laminin and 73.93% for fibronectin levels versus their corresponding values in the normal control. The present data demonstrated that TAA administration increases serum laminin level in rats. This result is in agreement with that of Yin *et al.* [35]. Laminins are major proteins in the basal lamina (one of the layers of the basement membrane), a

protein network foundation for most cells and organs. Laminin levels increase with the development of liver fibrosis [36]. TAA has been shown to develop liver fibrosis [37]. Thus, TAA could increase serum laminin level due to its ability to produce liver fibrosis.

The present data demonstrated that TAA increases serum fibronectin level in rats. Fibronectin plays a role in various biological processes such as fibrosis and tumor metastasis. Expression of NF-κB increased promoter activity in rat hepatocytes. NF-κB activates fibronectin gene expression by binding to the responsive element in rat hepatocytes [38]. Therefore, the oxidative stress and the generation of ROS due to TAA intoxication lead to activation of NF-κB with consequent activation of fibronectin gene expression and in turn fibronectin level.

Se is an important biological antioxidant, that is involved as a part of gluthation-SH-peroxidase, the selenoenzyme that catalyses reduction of lipid peroxides and hydrogen peroxide, thus prevents the nocive effects of lipid peroxidation. Selenium protects cells and cell membranes from oxidative processes, facilitating reaction between oxygen and hydrogen at membrane [39].

The present study showed that administration of two dose levels (0.94 and 0.47mg kg^{-1} b.w /day) of Se simultaneously with TAA caused significant decrease ($p<0.05$) in serum AST (-33.03% and -27.28% respectively) and ALT activities (-44.65% and - 33.58% respectively) as compared to TAA-intoxicated group (Table1). These results are in accordance with those of Naziroglu *et al.*, [40], who reported that Se mitigates the effect of liver disorders, protects the liver against hepatotoxic substance induced liver damage and decreases the elevation of serum AST and ALT activities caused by hepatocellular damage.

In the view of the obtained data, (Table 1) oral administration of two dose levels of Se simultaneously with TAA caused significant decrease ($p<0.05$) in serum ALP activity (21.19% and 13.70% respectively) as compared to TAA-intoxicated group .These results are in agreement with those of Shrivastava *et al.* [41].This finding might be attributed to the ability of Se to counteract free radical generation [42]. So, it could preserve the hepatocellular permeability and restore the elevated serum ALP level.

The obtained results revealed that administration of two dose levels of Se simultaneously with TAA caused significant decrease ($p<0.05$) in serum TNF-α, MCP-1 and IL-6 levels as compared to TAA-intoxicated group (Table 2). The percent of change for TNF-α (-17.12% for high Se dose and -10.15% for low Se dose). The percent of change for MCP-1 (-12.91% for high Se dose and -8.58% for low Se dose). The percent of change for IL-6 (-26.99% for high Se dose and -16.63% for low Se dose) as compared to TAA-intoxicated group.

Administration of two dose levels of Se simultaneously with TAA caused insignificant decrease ($p>0.05$) in serum IL-8 levels (-22.54% for high dose of Se and -15.15% for low dose of Se) as compared to TAA-intoxicated group (Table 2). Se, as an essential constituent of GPx, plays an important role in scavenging ROS [43] and has been known to regulate the activation of NF-κB [44]. Once NF-κB is inhibited, the gene expression of TNF-α, MCP-1, IL-6, IL-8 and fibronectin is in ordinately decreased. Consequently, serum levels of these cytokines decreased too. This mechanism explains the present result of the effect of Se administration on serum levels of TNF-α, MCP-1, IL-6, IL-8 and fibronectin in the treated rats. Treatment with Se afforded better protection, this may be due to the destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of damaged cell membrane [45]. Moreover, a growing body of evidence demonstrated that serum selenium levels are inversely correlated with serum concentrations of IL-8 [46]. Two possible explanations have been suggested: First, that the increased oxidative stress in liver disorder is caused by elevated IL-8 levels, which exhausts the available selenium that protects the cells exposed to inflammatory stress [46], and second, as shown by *in vitro* studies, that Se in the glutathione peroxidase system can inhibit IL-8 release by endothelial cells [47].

The data recorded in table (3) also showed significant decrease ($p<0.05$) in serum laminin and fibronectin for each of two dose levels of Se as compared to TAA-intoxicated group. The percent of change for laminin (-41.01% for high dose Se and -35.47% for low dose Se) and for fibronectin (-15.13% for high dose Se and -10.48% for low dose Se). Parsian *et al.* [36] reported that serum laminin level increases with the development of liver fibrosis. Se has been found to be effectively decreased the degree of hepatic fibrosis and promoted the recovery process [43]. By this way, Se could reduce serum laminin level in the treated rats. Se has been known to inhibit NF-κB [44] leading to decreasing the gene expression of fibronectin.

Brewer's and baker's yeast *(Saccharomyces cerevisiae)* has been used in classical food fermentation applications such as beer, bread, yeast extract/vitamins, wine, sake, and distilled spirits [48]. Hartwell *et al*. [9] have pioneered a

novel approach in which the yeast *Saccharomyces cerevisiae* is used to discover compounds with powerful chemotherapeutic potential.

The present finding showed that administration of two dose levels of yeast (*Saccharomyces cerevisiae*) (66.5 mg Kg^{-1} b.w and 33.25 mg Kg^{-1} b.w) simultaneously with TAA caused significant decrease ($p<0.05$) in serum AST and ALT activities as compared to TAA-intoxicated group (Table1). The percent of change for AST was -29.82% for high dose and -20.90% for low dose of yeast. The percentage of change for ALT was -40.12% for high dose and -31.56% for low dose of yeast. These results are in agreement with Darwish *et al.* [49], who reported that the pretreatment with *Saccharomyces cerevisiae* reduces serum AST and ALT activities and protects against hepatic injury caused by the mycotoxin.

In the view of the present data, administration of two dose levels of *Saccharomyces cerevisiae* simultaneously with TAA caused significant decreases ($p<0.05$) in serum ALP activity as compared to TAA intoxicated group. It was decreased by-19.66%for high dose and -11.00% for low dose of yeast. These results are in agreement with those of Mannaa *et al.* [14], who reported that the treatment with *Saccharomyces cerevisiae*, prior to clinical use of flutamide results in significant decrease in serum ALP activity due to the powerful active components in *Saccharomyces cerevisiae* which could modulate the severe hepatotoxicity caused by the reactive electrophilic metabolite 2-hydroxyflutamide and counteract the hard oxidative stress.

In the present study, administration of two dose levels of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA caused significant decrease ($p<0.05$) in serum TNF-α, MCP-1 and IL-6 levels as compared to TAA-intoxicated group. The percent of change for TNF-α (-17.41% for high yeast dose and -5.6% for low yeast dose). The percent of change for MCP-1 (-12.28% for high yeast dose and -10.28% for low yeast dose). The percent of change for IL-6 (-22.00% for high yeast dose and -14.31% for low yeast dose) as compared to TAA-intoxicated group. Administration of two dose levels of yeast simultaneously with TAA caused insignificant decrease ($p>0.05$) in serum IL-8 level (-22.16% for high dose of Se and -13.18% for low dose of Se) as compared to TAA-intoxicated group. High concentration of β-glucan resulted in suppression of TNF- α activity [50]. Also, simultaneous administration of two dose level of *Saccharomyces cerevisiae* with TAA significantly decreased serum IL-6 and IL-8 levels. These results are in agreement with those of Zanello *et al.* [51] who reported that viable *Saccharomyces cerevisiae* inhibits the expression of pro-inflammatory transcripts and proteins (IL-6 and IL-8). This inhibition was associated with a decrease of extracellular-signal-regulated kinases (ERK 1/2) and p38 mitogen-activated protein kinases (MAPK) phosphorylation. The present data demonstrated that administration of two dose level of *Saccharomyces cerevisiae* simultaneously with TAA significantly decreased serum MCP-1 level. The ROS have been known to increase the expression of chemoattractants such as MCP-1 [52]. However, it is also well reported that antioxidants can inactivate these ROS [53]. The polysaccharide beta-glucan, one of the major cell wall constituent of *Saccharomyces cerevisiae*, works like a free radical scavenger for ROS beside its activity as an antioxidant agent [54]. These roles of beta-glucan explain the effect of *Saccharomyces cerevisiae* on serum MCP-1 level in the treated rats.

Administration with two dose levels of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA resulted in significant decrease ($p<0.05$) in serum laminin and fibronectin as compared to TAA-intoxicated group (Table 3). The percent of change for laminin (-38.84% for high dose yeast and -32.02% for low dose yeast) and for fibronectin (-15.30% for high dose yeast and -11.89% for low dose yeast). The combined treatment with silymarine and β-glucan has been found to ameliorate or suppress fibrogenesis in the liver, protect liver cells from oxidative damage and stimulate regeneration of the parenchyma [55]. Therefore, β-glucan in *Saccharomyces cerevisiae* plays a potential role in the regression of fibrosis with consequent reduction in serum laminin level.

The present results revealed that administration of two dose levels of *Saccharomyces cerevisiae* simultaneously with TAA caused significant decrease in fibronectin level. NF-κB activates fibronectin gene expression by binding to the responsive element in rat hepatocytes [38]. Pretreatment or post treatment with glucans inhibited tissue NF-κB and tissue cytokines messenger RNA levels [56]. By this way, *Saccharomyces cerevisiae* could reduce serum fibronectin level in the treated rats.

Se in Se-Y is more biovailable than inorganic 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, a most interesting, useful and environmentally-safe supplementary material for use [57]. According to our results, administration of two dose levels of Se-Y (66.5mg Kg^{-1} b.w/ day and 33.25mg Kg^{-1} b.w/ day) simultaneously with TAA caused significant decrease ($p<0.05$) in serum AST and ALT activities as compared to TAA-intoxicated group (Table1). The percent of change for AST was -41.86 % for high dose and -24.56% for low dose of Se-Y. The percent of change for ALT was -54.03% for high dose and - 44.59%for low dose of Se-Y. Organic selenium has been found to decrease cytotoxicity and protect cells

from damage [58]. So, it could decrease the elevation of serum AST and ALT activities caused by hepatocellular damage as a result of TAA administration.

In the view of the obtained data (Table 1) oral administration of two dose levels of Se-Y simultaneously with TAA caused significant decrease ($p<0.05$) in serum ALP activity (-27.37% and -17.43% respectively) as compared to TAA-intoxicated group. This finding might be attributed to the ability of Se to counteract free radical generation [42]. So, it could preserve the hepatocellular permeability and restore the elevated serum ALP level. Organic Se, which exists in yeast products, primarily in the form of Se-methionine, resulted in a higher level of Se in animal products compared with inorganic Se [59].

Administration of two dose levels of Se-Y simultaneously with TAA caused significant decrease ($p<0.05$) in serum TNF-α, MCP-1, IL-6 and IL-8 levels as compared to TAA-intoxicated group (Table 2). The percent of change for TNF-α (-20.40% for high Se-Y dose and -5.2% for low Se-Y dose). The percent of change for MCP-1 (-19.54% for high Se-Y dose and -12.69% for low yeast dose). The percent of change for IL-6 (-27.95% for high Se-Y dose and -20.82% for low Se-Y dose). The percent of change for IL-8 (-35.03% for high Se-Y dose and -27.64% for low Se-Y dose) as compared to TAA-intoxicated group.

Oral administration with two dose levels of Se-Y simultaneously with TAA resulted in significant decrease ($p<0.05$) in serum laminin and fibronectin as compared to TAA-intoxicated group (Table 3). The percent of change for laminin (-48.36% for high dose Se-Y and -42.91% for low dose Se-Y) and for fibronectin (-29.35% for high dose Se-Y and -24.07% for low dose Se-Y).

The depletion of TNF-α, MCP-1, IL-6, IL-8 and fibronectin serum levels in rats treated with two dose levels of Se-Y (organic Se) simultaneously with TAA could be attributed to the action of organic Se through inactivation of NF-κB thereby reducing iNOS expression by reducing the level of ornithine decarboxylase activity and polyamine biosynthesis [60].

According to our results, administration with two dose levels of Se-Y simultaneously with TAA caused significant decrease in serum laminin level. Wasser *et al.* [61] reported that organic Se significantly causes the resolution of carbon tetrachloride-induced hepatic fibrosis in rats. Therefore, it could restore the level of laminin in serum.

Histopathological investigation

The microscopic examination of the liver tissue sections of normal control rats showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein (Figure 1A).

Supplementation with TAA for 6weeks showed necrosis with inflammatory cells infiltration which divided the hepatic parenchyma into nodules (Figure1B). These findings agree with the previous report of Anbarasu *et al.* [62] who reported that the liver sections of TAA treated animals showed hepatic cells with severe toxicity characterized by centrilobular necrosis along with various gradations of fatty changes comprising of tiny to large sized vacuoles.

Liver tissue sections of rats administered high dose of Se simultaneously with TAA for 6 weeks showed that there was fibroblastic cells proliferation and few inflammatory cells infiltration that originated from portal area and divided hepatic parenchyma into nodules associated with vesicular nuclei of the hepatocytes (Figure 1C) while, examination of liver tissue sections of rats administered low dose of Se simultaneously with TAA for 6weeks showed inflammatory cells infiltration with necrosis which originated from the portal area and extended to divided the hepatic parenchyma into nodules (Figure 1D). These results confirm the previous data of Shen *et al.* [43] who demonstrated that Se can also effectively decrease the degree of hepatic fibrosis and promote the recovery process [43].

Following the administration of high dose of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA for 6weeks revealed that there was inflammatory cells infiltration in the portal area as well as in few manners between the hepatocytes associated with vesicular nuclei of the hepatocytes (Figure 1E). While, examination of the liver tissue sections of rats supplemented with low dose of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA intoxication showed fibrosis with few inflammatory cells infiltration which divided the hepatic parenchyma into nodules (Figure 1F).These findings are in agreement with Mannaa *et al.* [14] who reported that in rats treated with both yeast and flutamide, the hepatic cords are more regularly arranged as compared to the flutamide-treated rats. Signs of apoptosis are less pronounced, and some hepatocytes appeared binucleated.

Liver tissue sections of rats administered high dose Se-Y simultaneously with TAA for 6weeks showed that there was dilatation in the central vein **(**Figure 1G). While, supplementation with low dose Se-Y simultaneously with

TAA for 6weeks showed fibrosis with collagen fibers proliferation and few inflammatory cells infiltration which divided the hepatic parenchyma into nodules **(**Figure 1H).

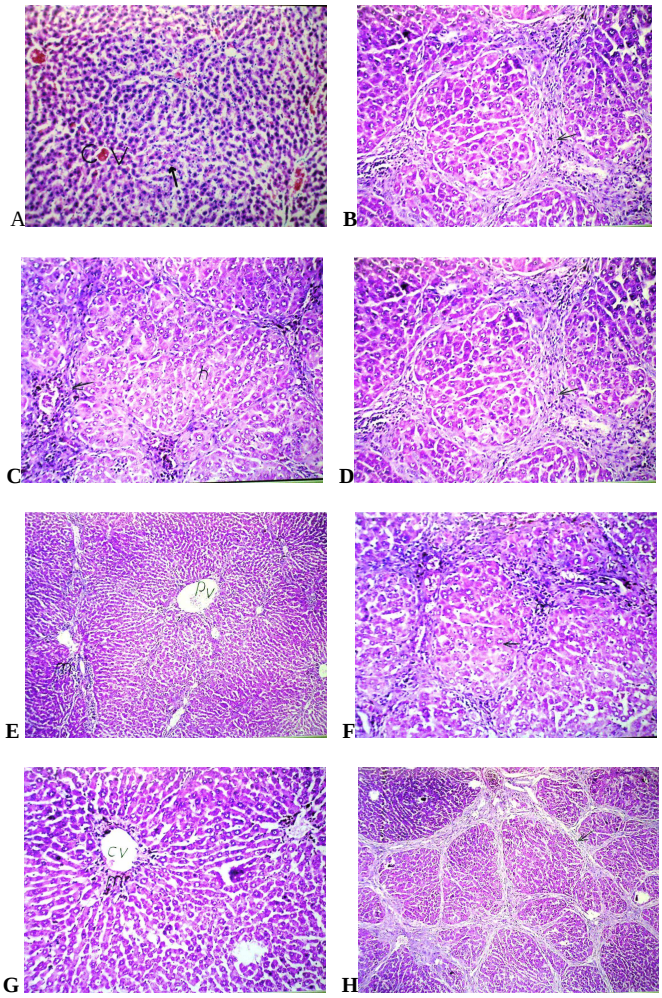

Figure 1: Photomicrographs of liver tissue sections of female rats in different studied groups. (A) Normal control showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. (B) TAA-intoxicated rat showed necrosis with inflammatory cells infiltration which divided the hepatic parenchyma into nodules. (C) High dose of Se+TAA showed fibroblastic cells proliferation and few inflammatory cells infiltration that originated from portal area and divided the hepatic parenchyma into nodules associated with vesicular nuclei of the hepatocytes. (D) Low dose Se + TAA showed inflammatory cells infiltration with necrosis that originated fromthe portal area and extended to divide the hepatic parenchyma into nodules. (E) High dose of *Saccharomyces cerevisiae* +TAA showed inflammatory cells infiltration in the portal area as well as in few manners between the hepatocytes associated with vesicular nuclei of the hepatocytes. (F)Low dose of *Saccharomyces cerevisiae* + TAA showed fibrosis with few inflammatory cells infiltration that divided the hepatic parenchyma into nodules. (H)High dose Se-Y+TAA showed dilatation in the central vein. (G) Low dose Se-Y+ TAA showed fibrosis with collagen fibers proliferation and few inflammatory cells infiltration that divided the hepatic parenchyma into nodules.

CONCLUSION

In conclusion, the selected doses of each of selenium, yeast and selenium-enriched yeast could protect the rat liver from thioacetamide-induced liver injury with special reference to selenium-enriched yeast which showed the most hepatoprotective effect. This could be due to that the organic selenium in the form of selenium-enriched yeast is more safe and efficient as hepatoprotective agent. Moreover, selenium enriched yeast exhibited antifibrotic and anti-inflammatory activities beside its antioxidant potential.

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