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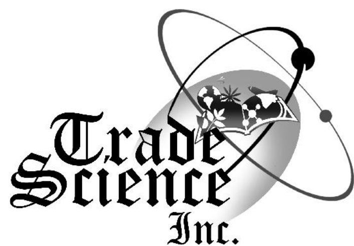

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Protective role of selenium-enriched yeast against hepatic necrosis induced by thioacetamide in experimental animals

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ABSTRACT

This study aimed at evaluating the protective role of Se-Y against TAA induced hepatic necrosis in rats. Sixty-four adult female rats were divided into 8 groups : (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 33.25 mg 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 33.25 mg kg^{-1} day^{-1}) respectively with simultaneous administration of TAA. Serum AST, ALT, ALP activities, hepatic SOD, CAT, GPx , eNOS activities, MDA and NO as well as eNOS activity. Histopathological examination of liver tissue sections was carried out. The results revealed that TAA intoxication caused significant increase in serum ALT, AST, ALP activities, hepatic MDA, NO levels and eNOS activity, while it produced significant decrease in hepatic SOD, CAT and GPx activities 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 hepatic necrosis as indicated by marked improvement in the studied biochemical markers and histopathological feature of liver tissue. In conclusion, Se, yeast and Se-Y could protect the rat liver from TAA-induced hepatic necrosis with special reference to Se-Y which showed the most pronounced effect. © 2013 Trade Science Inc. - INDIA

KEYWORDS

Hepatic necrosis;
Se;
Yeast;
Se-Y;
Rats.

INTRODUCTION

Hepatic injuries such as necrosis and fulminant hepatic failure (FHF), which are often produced on exposure of the tissue to virus or many chemical agents, constitute a major health hazard[1]. Liver necrosis and FHF are the two important clinical conditions of liver. Necrosis is a complex process, characterized by the simultaneous activation of multiple deregulated pathways that culminate in the loss of cell membrane integrity causing leakage of cellular constituents. FHF, on the other hand, is a rare but very lethal hepatic disorder characterized by an acute onset of severe hepatic dysfunction in the absence of preexisting liver disease

that results in jaundice, hepatic encephalopathy and coagulopathy[2].

Thioacetamide (TAA) is well known hepatotoxic agent, which produces hepatic necrosis by producing free radicals during TAA metabolism resulting in oxidative stress mediated acute hepatitis and induces apoptosis of hepatocytes in the liver[3]. It has been reported that long-term taken of TAA induces cirrhosis in rats. The acute hepatic necrosis induced by TAA, occurs through cytochrome p450 activation and a highly reactive compound called N-acetyle p-benzoquinone imine (NAPBQI) is produced which combines with sulphahydryl groups of proteins, causes a rapid reduction of intracellular glutathione, increases oxygen free radical causing an oxidative stress and initiates cellular apoptosis[4]. In addition, TAA could interfere with the movement of RNA from the nucleus to the cytoplasm and cause membrane injury resulting in a rise in serum liver markers[5]. Therefore, TAA toxic metabolite that induces oxidative stress in the hepatic cells is responsible for many changes occur for hepatocytes such as an increase in nuclear volume and enlargement of nucleoli, cell permeability changes, rise in intracellular concentration of Ca^{++}, and finally it affects mitochondrial activity leading to cell death[6]. Nowadays, there is an increased interest in dietary supplements with antioxidant properties that can reduce the deleterious effects of oxidative stress[7].

Selenium (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. Se protects cells and cell membranes from oxidative processes, facilitating reaction between oxygen and hydrogen and ions transfer at membrane level[8].

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[9]. Hartwell et al.[10] have pioneered a novel approach in which the yeast *Saccharomyces cerevisiae* is used to discover compounds with powerful chemotherapeutic potential. Remarkably, studies with *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[11].

The use of selenized yeast as enriched Se 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[12].

The present study was designed to assess the hepatoprotective activity of Se-Y against thioacetamide-induced hepatic necrosis in rats and to prove the scientific base of the traditional use of Se-Y against liver disorders.

MATERIALS AND METHODS

Materials

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).

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 pH 4.5. The yeast strain was maintained on the YEPD agar slants (2.5% agar). 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[13].

Preparation of selenium- enriched yeast

Se in inorganic form as sodium selenite (Na_2SeO_3) 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 19 μM of Se concentration in yeast is the proper concentration for achieving optimal growth of yeast cells[14]. These media with Se concentration was inoculated with 100 μl of overnight culture and incubated at 30°C for overnight growth on shaker[13].

Animals

Sixty-four adult female *Sprague-Dawley* 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 *ad libitum* access standard laboratory diet consisting of casein 10%, salts mixture 4 %,vitamins mixture 1%,corn oil 10 % and cellulose 5% completed to 100 g with corn starch[15]. 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). Group 1 served as normal control group. Group 2 was administered TAA (100 mg kg^{-1} b.w day^{-1})[16] 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 6 weeks. 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[14].

At the end of the experimental period, the rats were fasted overnight and subjected to diethyl ether anaesthesia. The blood samples were immediately withdrawn from the retroorbital 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 dried on filter paper. Each liver was divided into two portions, and the first portion was fixed in formalin saline (10%) for histopathological investigation and the second portion was weighed and homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containing 9.46 g di-sodium hydrogen phosphate and 9.07 sodium di-hydrogen phosphate (pH7.4). The homogenate was centrifuged at 1800 xg for 10 min in cooling centrifuge at 4 °C. The supernatant of each homogenate was stored at -80 till analysis.

Methods

Biochemical analyses

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured according to the method of Reitman and Frankel[17] and serum alkaline phosphatase activity (ALP) was estimated according to the method of Bowers and Mc Comb[18]. Hepatic superoxide dismutase (SOD) activity was detected according to the method of Nishikimi et al.[19]. Hepatic catalase (CAT) activity was determined according to the method of Aebi[20] and hepatic gluthathione peroxidase (GPx) activity was assayed according to the method of Paglia and Valentine[21]. Hepatic malondialdhyde (MDA) level was measured according to the method of Satoh[22], and hepatic nitric oxide level was determined according to the method of Montgomery and Dymock[23]. Hepatic endothelial nitric oxide synthase (eNOS) activity was assayed by ELIZA technique using eNOS assay kit purchased from Glory Science Co., New York, USA according to the manufactures instructions provided with eNOS assay kit.

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 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, and stained by hematoxylin and eosin stain. Then, examination was done through the light electric microscope[24].

Statistical analysis

All data were expressed as mean ± SE. Statistical analysis was done using the student t-test[25]. *P* values less than 0.05 were 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[4]. TAA is a well-established tool to induce hepatotoxicity specially necrosis in experimental animal models[26]. 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[27]. TAA is able to produce different grades of hepatic damage, including centrilobular necrosis[28]. 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.[29]. 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[30].

The data shown in TABLE 2 revealed a significant decrease ($p< 0.05$) of hepatic SOD, CAT and GPx activities in TAA-intoxicated group as compared to the control group. The percentage of decrement was -43.26% for SOD, -21.25% for CAT and -64.63% for GPx. Biological systems have protective arrangements which defend them against the harmful effects of free radicals. These include SOD and CAT[31]. In hepatotoxicity, these enzymes are structurally and functionally weakened by the radicals, resulting in liver

TABLE 1 : Effect of Se, yeast "Saccharomyces cerevisiae" and Se-Y on serum parameters of liver functions of TAA-intoxicated rats.

	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	56.99 ± 0.47	43.48 ± 0.85	107.38± 0.58
TAA (100 mg kg^{-1} b.w/day)	131.69 ± 1.45$^{\phi*}$(131.07%)	128.24 ± 1.93$^{\phi*}$(194.90%)	189.42 ± 0.90$^{\phi*}$(76.40%)
Se (0.96 mg kg^{-1} b.w/day)	88.18 ± 1.05$^{\bullet*}$(-33.03%)	70.97 ± 1.67$^{\bullet*}$(-44.65%)	149.27 ± 1.78$^{\bullet*}$(-21.19%)
Se (0.48 mg kg^{-1} b.w/day)	95.76 ± 0.96$^{\bullet*}$(-27.28%)	85.17 ± 2.01$^{\bullet*}$(-33.58%)	163.46 ± 2.77$^{\bullet*}$(-13.70%)
Yeast (66.5 mg kg^{-1} b.w/day)	92.42 ± 2.72$^{\bullet*}$(-29.82%)	76.78 ± 0.95$^{\bullet*}$(-40.12%)	152.18 ± 1.14$^{\bullet*}$(-19.66%)
Yeast (33.25 mg kg^{-}b.w/day)	104.16 ± 2.26$^{\bullet*}$(-20.90%)	87.76 ± 0.75$^{\bullet*}$(-31.56%)	168.37 ± 2.34$^{\bullet*}$(-11.00%)
Se-Y (66.5 mg kg^{-1} b.w/day)	76.56 ± 0.78$^{\bullet*}$(-41.86%)	58.95 ± 1.63$^{\bullet*}$(-54.03%)	137.57 ± 1.59$^{\bullet*}$(-27.37%)
Se-Y (33.25 mg kg^{-1}b.w/day)	99.34 ± 1.30$^{\bullet*}$(-24.56%)	71.05 ± 0.39$^{\bullet*}$(-44.59%)	156.40± 1.46$^{\bullet*}$(-17.43%)

ϕ & Difference in relation to control group. • Difference in relation to TAA-intoxicated group. * Significant change at $p< 0.05$.

TABLE 2 : Effect of Se, yeast "*Saccharomyces cerevisiae*" and Se-Y on hepatic antioxidant enzyme activities in TAA-intoxicated rats.

	SOD (U/g liver)	CAT (U/g liver)	GPx (U/g liver)
Normal control	399.67 ± 7.34	2375.66 ± 37.67	77.20 ± 1.69
TAA (100 mg kg^{-1} b.w/day)	226.77 ± 22.05 $^{\phi*}$(-43.26%)	1870.83 ± 14.13 $^{\phi*}$(-21.25%)	27.30 ± 0.63 $^{\phi*}$(-64.63%)
Se (0.96 mg kg^{-1} b.w/day)	331.56 ± 32.51 $^{\bullet*}$(46.21%)	2102.66 ± 28.63 $^{\bullet*}$(12.39%)	61.98 ± 1.47 $^{\bullet*}$(127.03%)
Se (0.48 mg kg^{-1} b.w/day)	281.39 ± 12.62 $^{\bullet*}$(24.08)	1998.00 ± 25.55 $^{\bullet*}$(6.79%)	48.64 ± 1.12 $^{\bullet*}$(78.16%)
Yeast (66.5 mg kg^{-1} b.w/day)	266.68 ± 28.86 $^{\bullet*}$(17.59%)	2114.66 ± 23.89 $^{\bullet*}$(13.03%)	47.64 ± 20.7 $^{\bullet*}$(74.50%)
Yeast (33.25 mg kg^{-}b.w/day)	250.86 ± 9.80 $^{\bullet*}$(10.62%)	2003.83 ± 25.12 $^{\bullet*}$(7.10%)	46.72 ± 1.91 $^{\bullet*}$(71.13%)
Se-Y (66.5 mg kg^{-1} b.w/day)	346.66 ± 25.66 $^{\bullet*}$(52.86%)	2244.00 ± 16.74 $^{\bullet*}$(19.94%)	37.80 ± 0.62 $^{\bullet*}$(38.46%)
Se-Y (33.25 mg kg^{-1}b.w/day)	306.52 ± 13.06 $^{\bullet*}$(35.16%)	2038.50 ± 20.71 $^{\bullet*}$(8.96%)	30.63 ± 1.47 $^{N.S}$(12.19 %)

• Difference in relation to TAA-intoxicated group. ϕ Difference in relation to control group.* Significant change at $p< 0.05$.

damage[32]. TAA could reduce the activity of CAT, which may cause the accumulation of O_2, H_2O_2, or their products of decomposition[33]. The present study also revealed a significant inhibition of hepatic GPx activity in TAA-intoxicated rats. This finding is in agreement with Abul et al.[34]. This result could be explained by the combination of the active metabolite of TAA, which is called NAPBQI, with the sulphahydryl groups of proteins causing rapid reduction of intracellular glutathione[4]. The reduction of intracellular glutathione leads to the inhibition of GPx as it is well known that glutathione is the substrate of GPx[35] and the activity of GPx is strictly linked with the concentration of glutathione (TABLE 2).

In the present study, administration of TAA in rats resulted in significant increase ($p< 0.05$) in hepatic MDA, NO levels and eNOS activity as compared to the normal control group. The percentage of increment was 87.84% for MDA, 86.99% for NO and 71.42% for eNOS (TABLE 3). Lipid peroxidation, which refers to the oxidative degradation of lipids, is a multifarious and natural harmful process[36]. The increased MDA level in the tissue represents an indirect index of lipid peroxidation. The increase in hepatic thiobarbituric acid reactive substance (TBARS) indicates enhanced lipid peroxidation which leads to tissue injury and failure of the anti-oxidant defense mechanisms to prevent the formation of excess free radicals[37]. The obtained data revealed that administration of TAA caused significant increase ($p< 0.05$) in hepatic MDA level as compared to the normal control group. This result might be attributed to the damaging impact effect of TAA which is known to induce hepatocyte damage following its metabolism to thioacetamide sulphene and sulphone, *via* a critical pathway involving cytochrome P450-mediated biotransformation[38]. These metabolites are highly reactive and thus they lead to the denaturation of cellular biomolecules such as lipids, resulting in lipid peroxidation and its byproduct[39]. In the present study, administration of TAA caused significant increase ($p< 0.05$) in hepatic NO level and eNOS activity as compared to the normal control group. Several reports indicated that exposure of cells to H_2O_2 promotes eNOS expression and thus NO synthesis[40]. Chen et al.[41] reported that there is a significant production of H_2O_2 in TAA- induced toxicity.

The present study showed that administration of two dose levels (0.94 and 0.47 mg 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 (TABLE 1). These results are in accordance with those of Naziroglu et al.[42], 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.[43]. This finding might be attributed to the ability of Se to counteract free radical generation[44]. So, it could

TABLE 3 : Effect of Se, yeast "Saccharomyces cerevisiae" and Se-Y on oxidative biomarkers of the liver of TAA-intoxicated rats.

	MDA (nmol/ g liver)	NO(µmol / g liver)	eNOS (U/ g liver)
Normal control	38.58 ± 0.68	33.30 ± 0.42	1.96 ± 0.03
TAA (100 mg kg^{-1} b.w/day)	72.47 ± 1.41 $^{\phi*}$(87.84%)	62.27 ± 0.85$^{\phi*}$(86.99%)	3.36 ± 0.15$^{\phi*}$(71.42%)
Se (0.96 mg kg^{-1}b.w/day)	47.34 ± 0.54 $^{\bullet*}$(-34.76%)	39.75 ± 0.80$^{\bullet*}$(-36.16%)	2.59 ± 0.08 $^{\bullet*}$(-22.91%)
Se (0.48 mg kg^{-1}b.w/day)	54.97 ± 1.36 $^{\bullet*}$(-24.14%)	43.28 ± 0.39$^{\bullet*}$(-30.49%)	3.27 ± 0.04 $^{N.S}$(-2.67%)
Yeast (66.5 mg kg^{-1}b.w/day)	45.04 ± 0.84 $^{\bullet*}$(-37.85%)	40.03 ± 1.49$^{\bullet*}$(-35.71%)	2.64 ± 0.07 $^{\bullet*}$(-21.43%)
Yeast (33.25 mg kg^{-}b.w/day)	46.32 ± 0.60 $^{\bullet*}$(-36.08%)	45.55 ± 1.16$^{\bullet*}$(-26.85%)	3.35 ± 0.04 $^{N.S}$(-0.29%)
Se-Y (66.5 mg kg^{-1}b.w/day)	42.38 ± 0.60 $^{\bullet*}$(-41.52%)	36.63 ± 1.83$^{\bullet*}$(-41.17%)	2.25 ± 0.04 $^{\bullet*}$(-33.04%)
Se-Y (33.25mg kg^{1}b.w/day)	46.39 ± 0.52 $^{\bullet*}$(-35.98%)	39.11 ± 1.09$^{\bullet*}$(-37.19%)	2.67 ± 0.07 $^{\bullet*}$(-20.53%)

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

preserve the hepatocellular permeability and restore the elevated serum ALP activity.

The obtained results revealed that administration of two dose levels of Se simultaneously with TAA caused significant increase ($p< 0.05$) in hepatic SOD, CAT and GPx activities as compared to TAA-intoxicated group (TABLE 2). The percent of change for SOD activity was (46.21% for high Se dose and 24.08% for low Se dose). The percent of change for CAT activity was (12.39% for high Se dose and 6.79% for low Se dose). The percent of change for GPx activity was (127.03% for high Se dose and 78.16% for low Se dose). The increase in hepatic SOD and GPx activities could be attributed to the role of Se in binding the active sites of SOD and GPx[45]. Se plays an important role in the development and maintenance of the antioxidant defense system[46]. Whereas, the significant elevation of hepatic CAT activity higher than that in TAA-intoxicated group reflect most probably, the adaptive response towards the damaging impact of free radicals in the liver, as reported by El Heni et al.[47]. The effect of Se in enhancing hepatic GPx activity might be attributed to the increased bioavailability of Se following treatment with sodium selenite[48].

In the view of the obtained data, oral administration of two dose levels of Se simultaneously with TAA caused significant decrease ($p< 0.05$) in hepatic MDA level as compared to TAA-intoxicated group (TABLE 3). It was decreased by -34.67% and -24.14% respectively. This result is in agreement with Sodhi et al.[49], who reported that the protective effect of α-tocopherol and Se on liver resulted from their antioxidant activity which causes stabilization in the intracellular defense systems and reduction in lipid peroxidation products and the production of reactive oxygen species.

In the present study, administration with high dose Se (0.94 mg Kg^{-1} b.w simultaneously with TAA caused significant decrease ($p< 0.05$) in hepatic NO level and eNOS activity as compared to TAA-intoxicated group. The percent of change for NO was -36.16% and for eNOS was -22.91%. While, administration with low dose Se (0.48 mg Kg^{-1} b.w) simultaneously with TAA caused significant decrease ($p< 0.05$) in hepatic NO level accompanied with insignificant change ($p> 0.05$) in hepatic eNOS activity as compared to TAA-intoxicated group. The percent of change for NO was -30.49% and for eNOS was -2.67% (TABLE 3). The exact mechanism by which Se influence hepatic NO synthase expression is unknown. Of interest in this context is the report that treatment of nuclear extracts of lipopolysaccharide-activated human T cells with relatively high concentrations of selenite inhibited nuclear factor-κB binding and thus decreased NO production[50]. This is because nuclear factor-κB is a transcription factor that regulates a number of cellular genes, such as those encoding eNOS[51].

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 (TABLE 1). 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.[52], 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.[53] 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 with two dose levels of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA caused significant increase ($p< 0.05$) in hepatic SOD, CAT and GPx activities as compared to TAA-intoxicated group (TABLE 2). The percent of change for SOD was 17.59% for high dose and 10.62% for low dose of yeast. The percent of change

for CAT was 13.03% for high dose and 7.10% for low dose of yeast. The percent of change for GPx was 74.50% for high dose and 71.13% for low dose of yeast. It has been reported that feeding rabbits with glucomannan one of the active components of *Saccharomyces cerevisiae* cell wall, causes an increase in SOD activity and GSH contents of the liver[54]. The aforementioned studies showed that β-D-glucan increased the activities of antioxidant enzymes, such as SOD, CAT, GPx and GSH, and inhibited lipid peroxidation[55].

A selenoprotein, GPx provides a useful tool for investigating the functional Se-status in animals[56]. For several years, GPx was thought to be the only known selenoprotein in mammalian tissues. A portion of the yeast selenomethionine is metabolized to selenocysteine which is then decomposed to selenide by selenocysteine β-lyase. This selenide-Se fraction is incorporated into the GPx molecule[57]. In this way, we can interpret the observed marked increase observed in hepatic GPx activity of rats treated with *Saccharomyces cerevisiae* simultaneously with TAA.

According to our results, administration with two dose levels of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA caused significant decreases ($p< 0.05$) in hepatic MDA level as compared to TAA-intoxicated group, it was decreased by -37.85% and -36.08% respectively (TABLE 3). Krizkova et al.[58] stated that *Saccharomyces cerevisiae* cell wall mannan has relatively good antioxidative effects as it is capable to scavenge reactive oxygen radicals. Masson and Ramotar[59] proposed that the *Saccharomyces cerevisiae* c-IMP2 gene prevents oxidative damage by regulating the expression of genes that are directly required to repair DNA damage.

In the present study, administration of high dose of *Saccharomyces cerevisiae* simultaneously with TAA caused significant decrease ($p< 0.05$) in hepatic NO level and eNOS activity as compared to TAA-intoxicated group. The percent of change for NO was -35.71% and for eNOS was -21.43%. While, administration of low dose *Saccharomyces cerevisiae* simultaneously with TAA caused significant decrease ($p< 0.05$) in hepatic NO level accompanied with insignificant alteration ($p>0.05$) in hepatic eNOS activity as compared to TAA-intoxicated group (TABLE 3). The percent of change was -26.85% for NO and -0.29% for eNOS. The exposure of cells to H_2O_2 promotes eNOS expression and thus NO synthesis[59]. The polysaccharide beta-glucan, one of the major cell wall constituent of *Saccharomyces cerevisiae*, works like a free radical scavenger and has an antioxidant effect on lipid peroxidation[60]. Thus, it could reduce H_2O_2 production and hence, it inhibits eNOS activity and NO generation.

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 (66.5 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 (TABLE 1). 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[61]. 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[44]. 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[62].

The obtained results revealed that administration of two dose levels of Se-Y simultaneously with TAA caused significant increase ($p<0.05$) in hepatic SOD and CAT activities while it caused significant decrease ($p<0.05$) in hepatic MDA level as compared to TAA-intoxicated

group. The percent of change for SOD was 52.86% for high dose and 35.16% for low dose of Se-Y. The percent of change for CAT was 19.94% for high dose and 8.96% for low dose of Se-Y. The percent of change for MDA was -41.52% for high dose and -35.98% for low dose of Se-Y (TABLE 2 and 3). These results are in agreement with Ahmad et al.[63] who reported that the administration of Se-Y significantly increased the activities of SOD and CAT, but decreased the MDA content in chickens.

The present study showed that administration of high dose level of Se-Y simultaneously with TAA caused significant increase ($p<0.05$) in hepatic GPx activity (38.46%) as compared to TAA-intoxicated group while administration of low dose level of Se-Y simultaneously with TAA displayed nearly no change ($p>0.05$) in hepatic GPx activity (12.19%) as compared to TAA-intoxicated group (TABLE 2). This result is in agreement with Chung et al.[64], who reported that goats fed the diet containing organic Se showed significant increase in GPx activity compared with those fed the basal diet. Increased GPx activity suggests that the dietary organic Se may have a positive effect on the antioxidant defense system of goats[64].

In the present study, administration with two dose levels of Se-Y simultaneously with TAA caused significant decrease ($p<0.05$) in hepatic NO level and eNOS activity as compared to TAA-intoxicated group (TABLE 3). The percent of change for NO was -41.17% for high dose and -37.19 for low dose Se-Y. The percent of change for eNOS was -33.04% for high dose and -20.53 for low dose Se-Y. These results are in agreement with Das and Battacharya[65], who stated that significant decrease of NO level was observed in organic selenium treatment groups indicating its role in reducing the generation of NO by inhibiting the expression of iNOS mediated excessive production of NO and its conversion to strong electrophillic intermediates $ONOO^-$ and N_2O_3.

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 6 weeks showed necrosis with inflammatory cells infiltration which divided the hepatic parenchyma into nodules (Figure 1B). These findings agree with the previous report of Anbarasu et al.[66] 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 6 weeks 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.[67] who demonstrated that Se can also effectively decrease the degree of hepatic fibrosis and promote the recovery process[67].

Following the administration of high dose of yeast (*Saccharomyces cerevisiae*) simultaneously with TAA for 6 weeks 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.[53] 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 6 weeks showed that there was dilatation in the central vein (Figure 1G). While, supplementation with low dose Se-Y simultaneously with TAA for 6 weeks showed fibrosis with collagen fibers proliferation and few inflammatory

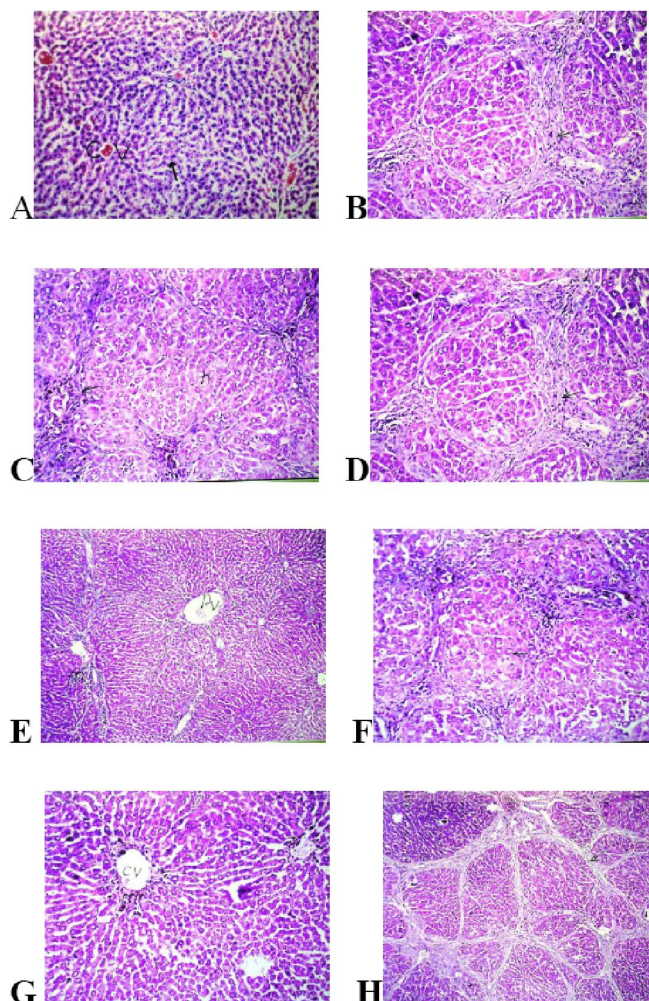

Figure 1 : Photomicrographs of liver tissue sections of female rats in different studied groups. (A) Normal control showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. (B) TAA-intoxicated rat showed necrosis with inflammatory cells infiltration which 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 from the 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.

cells infiltration which divided the hepatic parenchyma into nodules (Figure 1H).

ABBREVIATIONS

Selenium (Se), Selenium-enriched yeast (Se-Y), Thioacetamide (TAA), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Malondialdhyde (MDA), Nitric oxide (NO), endothelial nitric oxide synthase (eNOS), Fulminant hepatic failure (FHF), N-acetyle p-benzoquinone imine (NAPBQI), Thiobarbituric acid reactive substance (TBARS).

CONCLUSION

The current study clarified that the selected high doses of selenium, yeast (*Saccharomyces cerevisiae*) and selenium enriched yeast have hepatoprotective effect against thioacetamide-induced hepatic necrosis. Special attention should be given to selenium-yeast which showed the most pronounced hepatoprotective efficacy.

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