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Potential Ameliorative Effects of Alpha Lipoic Acid and Silymarin on Thioacetamide-Induced Hepatic Damage in Rats

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ABSTRACT

The present study was designed to study the ameliorative role of alpha lipoic acid (ALA) and silymarin (SIL) against thioacetamide (TAA)-induced hepatic damage. Sixty male albino rats were randomly separated into 6 groups (n=10); control group, TAA-treated group: received intraperitoneal injection of TAA 250 mg/kg bwt/day three times a week, ALA-treated group: received ALA 100 mg/kg bwt/day) orally three times a week, SIL-treated group: received SIL 100 mg/kg bwt/day orally three times a week, TAA+ALA-treated group, and TAA+SIL-treated group at the same previous doses and routes for ten weeks.
Results showed that TAA induced a significant elevation in total and direct bilirubin, total cholesterol and triglycerides levels and serum liver enzymes after four and ten weeks of the treatments. While the serum levels of total proteins, albumin and high density lipoprotein-cholesterol revealed a significant decrease. TAA injection resulted in an increase in the hepatic lipid peroxidation and decreased levels of antioxidant biomarker. Histopathologically, TAA revealed marked degenerative, necrotic and fibrotic alterations in the liver, particularly during ten weeks post-treatment. ALA and SIL- treatment ameliorated TAA-induced oxidative damage, alterations in the liver function tests and liver histopathology. However, ALA demonstrated better hepatic protection against TAA-induced liver damage as compared to SIL. The study clearly concluded that ALA has more powerful antioxidant properties than SIL.

1. INTRODUCTION

Hepatotoxins can react with the main cellular components inducing various hepatic alterations (Grattagliano et al., 2009). Thioacetamide (TAA) is a well-recognized hepatocarcinogen that induces oxidative damage in hepatocytes through generation of reactive oxygen species (ROS), and subsequent liver cirrhosis and liver cell tumors in rodents (Uskoković-Marković et al., 2007). Thus, it is widely used to induce acute toxic hepatic damage through oxidative stress, membrane damage and accumulation of lipid droplets in the hepatocyte cytoplasm (Koen et al., 2013). Alpha lipoic acid (ALA), also named as thiotic acid, is a natural cofactor that is synthesized inside the plant and animal mitochondria (Thaakur and Himabindhu, 2009). ALA has recently used as a protective agent for many pathological alterations that are due to oxidative stress (Li et al., 2012 and Wang et al., 2013). The antioxidant activity of ALA may discussed by its ability to scavenge free radicals in both lipophilic and hydrophilic environments, and to restore endogenous antioxidants, chelate transition metals, decrease lipid oxidation, prevent protein glycation and repair oxidatively damaged biomolecules (Palaniyappan and Alphonse, 2011). Several investigations have concluded the efficacy of ALA treatment in the prevention of pathologic conditions mediated by oxidative stress (Castro et al., 2013). Silymarin (SIL) is an extract of the milk thistle (*Silybum marianum*) and it is considered as a standard supportive drug exhibiting a potent hepatoprotective activity for hepatic diseases of various causes through its antioxidant activity, stimulates protein synthesis, affects lipid metabolism, and stabilizes membrane phospholipids (Nencini et al., 2007 and El-Shitany et al., 2008). Thus, the current investigation was designed to evaluate the hepatoprotective efficacy of both ALA and SIL against TAA-induced hepatotoxicity.

1. MATERİALS AND METHODS

2.1. Chemicals

Thioacetamide (TAA) was purchased from Biotechnology Company, Cairo, Egypt. Alpha-lipoic acid (Thiotacid®) tablets were manufactured by EVA pharma for pharmaceuticals and Medical Appliances, Egypt. SIL was obtained as a pure material from Pharaonic Pharmaceutical Company, Egypt.

2.2. Animals and experimental design

Sixty apparently healthy adult male albino rats (140 – 160 g bwt 10 weeks of age), were purchased from a closed random bred colony at the Medical Research Institute of Alexandria University, Egypt. Rats were kept in separated clean metal cages with free access to food and drinking water. All rats were allowed two weeks as acclimatized period before the beginning of study for adaptation and ensure normal growth and behavior. Rats were randomly separated into 6 groups (10 rats each): control group: received 0.2 ml of distilled water/ 100 g bwt/ day orally (vehicle of TAA and ALA) using a stomach tube three times a week, TAA-treated group: received intraperitoneal (IP) injection of TAA (250 mg/kg bwt dissolved in distilled water) three times a week according to Abdou et al. (2015). ALA-treated group: received ALA (100 mg/kg bwt/day) orally using a stomach tube three times a week according to Yousef et al., (2015). SIL-treated group: received SIL (100 mg/kg bwt/day) orally three times a week according to El-Senosiy et al. (2015). TAA+ALA-treated group: received IP injected with TAA (250 mg/kg bwt) and ALA given orally (100 mg/kg bwt). TAA+ SIL-treated group: injected intraperitoneally with TAA (250 mg/kg bwt) and SIL administered orally (100 mg/kg bwt). All experimental procedures were continued for ten weeks.

2.3. Blood sampling

Blood samples were collected from the retro-orbital venous plexus of each rat before being euthanized under light ether anesthesia. The blood samples (about 5 ml of blood) were placed in plain centrifuge tubes, left in slope position at room temperature to clot and centrifuged for 15 at 3000 rpm minutes for separation of serum. The clear serum was carefully separated, then transferred into clean dry epindorffs and kept frozen at –20°C until used for biochemical analysis.

2.4. Serum biochemical parameters

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (kits from Bio-labo, France) were measured calorimetrically according to the previously described method (Reitman and Frankle, 1957). Serum alkaline phosphatase (ALP) activity was measured kinetically according to Belfield and Goldberry (1971). Serum gamma glutamyle transferase (GGT) activity was estimated by kinetic method according to Szasz (1974). The level of serum total proteins, serum albumin, serum total and direct bilirubin, serum total cholesterol (TC) and triglycerides (TG), High density lipoprotein-cholesterol (HDL) were determined according to the previously described methods (Doumas et al., 1981; Doumas and Biggs, 1972; Walter and Gerade, 1970; Friedman and Young, 1997; Burstein et al., 1980; respectively) using commercial diagnostic kits supplied by Vitro Scient. Co. Egypt

2.5. Antioxidant status and oxidative stress assays

Small piece of liver of each rat was kept frozen at – 70°C for assessment of lipid peroxidation (LPO) contents, reduced glutathione (GSH) concentration and activity of glutathione S-transferase (GST). LPO was quantified as malondialdehyde (MDA) according to the previously described method (Ohkawa *et al.*, 1979). Moreover, spectrophotometrical assessment of GSH level was performed according to the method described by Sedlak and Lindsay (1968). However, the level of GST activity was determined according to the method of Habig et al. (1974). Kits obtained from Bio-diagnostic co. Egypt.

2.6. Histopathologic studies

Following necropsy, small specimens of liver were collected from control and treated rats, and rapidly fixed in 10% neutral formalin for 24 hrs. After fixation, tissue specimens were processed through the conventional paraffin embedding technique. 5 µm thick sections were obtained from paraffin blocks, stained with hematoxylin and eosin (HE) (Bancroft et al., 2013) and examined under light microscope.

2.7. Statistical analysis

Data were statistical analyses using the Statistical Analysis System software (SAS, 2011). Effect of treatments on biochemical was performed by the analysis of variance. Means were compared using Duncan's Multiple Range test at a significance level of $P \leq 0.05$. Values are presented as means $\pm$standard errors.

3. RESULTS

3.1. Serum biochemical assays

As recorded in Table (1), the serum ALT activity showed a significant increase in both TAA- and TAA+SIL-treated groups. While, the serum activity of AST, ALP and GGT were significantly

increased in TAA-, TAA+ALA-and TAA+ SIL-treated rats compared with control rats at four weeks post-treatment. At ten weeks post-treatment, the serum activity of ALT, AST and GGT showed significant increase in TAA-, TAA+ALA- and TAA+ SIL-treated groups. However, serum ALP activity was significantly elevated in TAA-treated group only compared to control group. The greatest increase of serum liver enzymes was reported in TAA-treated rats followed by TAA+ SIL then TAA+ALA treated rats at ten weeks post-treatment. However, serum total proteins level showed a significant reduction in TAA-treated group while, serum albumin level showed a significant decrease in TAA-, TAA+ ALA- and TAA+ SIL -treated groups at four weeks post-treatment compared to control group. At ten weeks post-treatment, serum total protein, albumin level showed a significant decrease in TAA-treated group. The highest reduction was observed in TAA-treated group comparatively to other groups. The serum total and direct bilirubin showed a significant increase in TAA-, TAA+ALA- and TAA+ SIL-treated groups at four and ten weeks post-treatment. The marked increase was observed in TAA-treated group at four weeks post-treatment compared to control. Significant increase in serum TC and TG was reported in TAA-treated rats at four weeks post-treatment while at ten weeks TC displayed a significant increase in TAA-, TAA+ALA- and TAA+ SIL-treated groups. Moreover, serum TG was significantly increased in TAA- and TAA+ALA- treated rats. The highest increase was observed in TAA-treated rats. On the other hand, serum HDL was significantly decreased in TAA-treated rats at four and ten weeks post-treatment. TAA+ALA- and TAA+ SIL-treated groups showed no significant changes in serum HDL during the first 4 weeks and significant elevation at the end of the experiment.

3.2. Antioxidant status and oxidative stress assays

As illustrated in Table (2), four weeks of the treatment resulted in significant increase in the hepatic MDA values and a significant decrease in the concentration of hepatic GSH and GST in TAA-treated group as compared to control group. Regarding to the TAA+ ALA-treated group, no significant changes in MDA, GSH and GST concentrations were recorded. Moreover, TAA+ SIL treated-group did not induce any significant alteration in MDA and GSH values, but GST concentration was significantly decreased compared to control. Ten weeks post-treatment the concentration of hepatic MDA showed a significant increase in TAA-treated group while, the concentration of hepatic GSH and activity of GST showed a significant decrease in TAA-treated group compared to control group. No significant changes were detected in other treated groups.

3.3. Histopathologic results:

Liver of the control group, ALA- and SIL-treated groups exhibited normal histological appearance at the two time points of examination (four and ten weeks) (Fig. 1a). At four weeks post-treatment, liver of euthanized TAA-treated rats showed centrilobular, periportal or diffuse cytoplasmic vacuolation of the hepatocytes of hydropic type (Fig. 1b), portal areas showed moderate to great thickening by mononuclear cells infiltration and biliary epithelial hyperplasia that was represented by papillary folding or formation of newly formed bile ductules (Fig. 1c). Ten weeks post treatment liver of TAA-treated rats exhibited periportal to diffuse hepatocytic vacuolation of fatty type, focal to multifocal areas of hepatic necrosis that were infiltrated by mononuclear inflammatory cells. Larger areas of zonal hepatic necrosis were extended to involve the neighboring lobule forming a thin necrotic tract (bridging necrosis) (Fig. 1d). In addition, the portal areas were markedly thickened and extended giving the nodular appearance (Fig. 1e). Moreover, apoptotic cells within the hepatic parenchyma were noticed (Fig. 1f). During the first four weeks, the co-treatment of ALA with TAA showed hepatoprotective effect as portal areas were slightly or moderately thickened by mononuclear cells infiltration (Fig. 2a). At ten weeks post-treatment, there were mild pathological alterations such as mild congestion and mononuclear cell infiltrations inside the portal triads (Fig. 2b), with mild focal hepatic necrosis and mononuclear cell infiltrations. At the four weeks post-treatment, diffuse hepatocytic vacuolation of the hydropic type was noticed (Fig. 3a). Moderate thickening of portal area with mononuclear inflammatory cells, biliary epithelial hyperplasia and mild fibroplasia (Fig. 3b) were evident at the ten weeks of the experiment. Also, SIL administration ameliorated the TAA-induced pathological lesions in the liver of euthanized rats wherein, the hepatic lesions were more severe lesions than those reported in TAA+ALA-treated rats, but lesser in the severity than those of the TAA-treated rats.

Table 1: Effect of thioacetamide (TAA), alpha lipoic acid (ALA) and silymarin administration alone or in combination for four and ten weeks on serum biochemical parameters in male albino rats.

		Groups					
Parameter	Week	control	TAA	ALA	Silymarin	TAA+ALA	TAA+ silymarin
ALT (U/L)	4	30.14 ± 1.13c	46.98 ± 1.25a	30.26 ± 1.82c	30.40 ± 1.80c	33.02 ± 1.79c	39.00 ± 1.62b
	10	26.14 ± 1.56d	47.88 ± 1.97a	29.22 ± 1.33cd	30.92 ± 1.75cd	36.32 ± 1.19b	33.92 ± 1.54bc
AST (U/L)	4	50.42 ± 2.83c	117.00 ± 4.67a	52.52 ± 2.72c	49.00 ± 3.19c	97.50 ± 3.25b	100.10 ± 2.98b
	10	79.90 ± 3.18d	157.14 ± 5.67a	83.32 ± 2.89d	82.10 ± 3.71d	103.74 ± 4.00c	120.86 ± 5.68b
ALP (U/L)	4	99.08 ± 3.81c	227.60 ± 5.31a	105.92 ± 4.74c	107.28 ± 4.61c	160.00 ± 3.45b	165.74 ± 4.62b
	10	180.80 ± 6.15b	267.20 ± 7.97a	182.00 ± 4.67b	182.00 ± 5.22b	187.40 ± 7.33b	185.52 ± 7.72b
GGT (U/L)	4	20.78 ± 1.45c	36.58 ± 2.54a	24.40 ± 1.75bc	24.92 ± 3.36bc	31.50 ± 1.80ab	34.00 ± 3.65a
	10	26.80 ± 3.22c	49.06 ± 3.67a	31.38 ± 3.39bc	32.04 ± 2.11bc	38.60 ± 3.89b	40.60 ± 2.25ab
Total protein (g/dl)	4	6.52 ± 0.015a	5.36 ± 0.17b	6.46 ± 0.24a	6.50 ± 0.22a	5.96 ± 0.17ab	6.02 ± 0.30ab
	10	7.64 ± 0.17a	5.46 ± 0.18b	7.42 ± 0.19a	7.46 ± 0.23a	7.20 ± 0.27a	7.42 ± 0.26a
Albumin (g/dl)	4	3.54 ± 0.10a	2.08 ± 0.16d	3.40 ± 0.15ab	3.42 ± 0.15ab	2.90 ± 0.19c	2.98 ± 0.18bc
	10	3.50 ± 0.23a	2.04 ± 0.09b	3.34 ± 0.14a	3.40 ± 0.18a	3.18 ± 0.18a	3.10 ± 0.18a
Total bilirubin (mg/dl)	4	0.45 ± 0.02c	0.70 ± 0.03a	0.47 ± 0.03c	0.46 ± 0.03c	0.56 ± 0.03b	0.63 ± 0.03ab
	10	0.39 ± 0.03c	0.68 ± 0.03 a	0.38 ± 0.03c	0.38 ± 0.03c	0.54 ± 0.03b	0.49 ± 0.04b
Direct bilirubin (mg/dl)	4	0.12 ± 0.02 c	0.36 ± 0.04a	0.14 ± 0.02c	0.13 ± 0.02c	0.22 ± 0.03b	0.28 ± 0.02ab
	10	0.09 ± 0.02c	0.34 ± 0.02a	0.09 ± 0.02c	0.10 ± 0.02c	0.20 ± 0.02b	0.18 ± 0.02b
Total cholesterol (mg/dl)	4	105.80 ± 2.29bc	123.60 ± 1.96a	112.00 ± 3.48b	102.60 ± 3.25c	113.20 ± 2.29b	101.40 ± 2.80c
	10	73.86 ± 2.54c	98.02 ± 1.82a	74.68 ± 2.55c	75.68 ± 2.32c	87.36 ± 2.15b	86.96 ± 2.46b
Triglycerides (mg/dl)	4	83.20 ± 5.10b	123.40 ± 5.80a	85.80 ± 4.19b	84.40 ± 4.13b	83.20 ± 5.94b	88.20 ± 5.13b
	10	84.06 ± 2.08c	122.50 ± 2.34a	86.12 ± 2.21c	85.68 ± 1.80c	93.40 ± 2.32b	86.08 ± 2.25c
HDL (mg/dl)	4	32.18 ± 2.30a	20.46 ± 1.44b	28.60 ± 1.63a	29.80 ± 2.08a	29.60 ± 1.81a	28.20 ± 2.76a
	10	16.86 ± 2.54c	50.80 ± 3.36a	21.12 ± 3.54c	21.44 ± 3.81c	32.80 ± 3.84b	33.80 ± 3.45b

TAA = Thioacetamide; ALA = Alpha-lipoic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, Gamma glutamyle transferase.
HDL= high density lipoprotein.
Values are means ± standard errors. Means with different letter within the same row differ significantly ($P<0.05$).

Table 2: Effect of thioacetamide (TAA), alpha lipoic acid (ALA) and silymarin administration alone or in combination for four and ten weeks on hepatic lipid peroxidation and antioxidant biomarkers concentration in albino male rats.

		Groups					
Parameters	Week	control	TAA	ALA	silymarin	TAA+ALA	TAA+ silymarin
MDA (nmol /g wet tissue)	4	7.00 ± 1.15b	14.33 ± 2.60 a	8.00 ± 1.73b	6.33 ± 1.33b	5.67 ± 1.76b	7.00 ± 1.53b
	10	8.33 ± 1.33b	16.00 ± 2.89a	6.33 ± 1.45b	6.67 ± 0.88b	7.33 ± 1.76b	4.67 ± 1.67b
GSH (µmol/ g wet tissue)	4	4.10 ± 0.29a	2.40 ± 0.38b	4.10 ± 0.52a	3.97 ± 0.57a	3.97 ± 0.58a	3.47 ± 0.37ab
	10	3.67 ± 0.43a	2.10 ± 0.29b	3.60 ± 0.35a	3.23 ± 0.68ab	3.50 ± 0.32ab	3.20 ± 0.38ab
GST (U/ g wet tissue)	4	0.28 ± 0.04a	0.06 ± 0.02c	0.31 ± 0.03a	0.24 ± 0.04a	0.26 ± 0.05a	0.19 ± 0.04b
	10	0.23 ± 0.03a	0.07 ± 0.03b	0.21 ± 0.03a	0.17 ± 0.04ab	0.20 ± 0.03a	0.17 ± 0.03ab

TAA = Thioacetamide; ALA = Alpha-lipoic acid; GSH=Reduced glutathione, MDA= Malondialdhyde, GST= glutathione-s-transferase. Values are means ± standard errors. Means with different letter within the same row differ significantly (P<0.05).

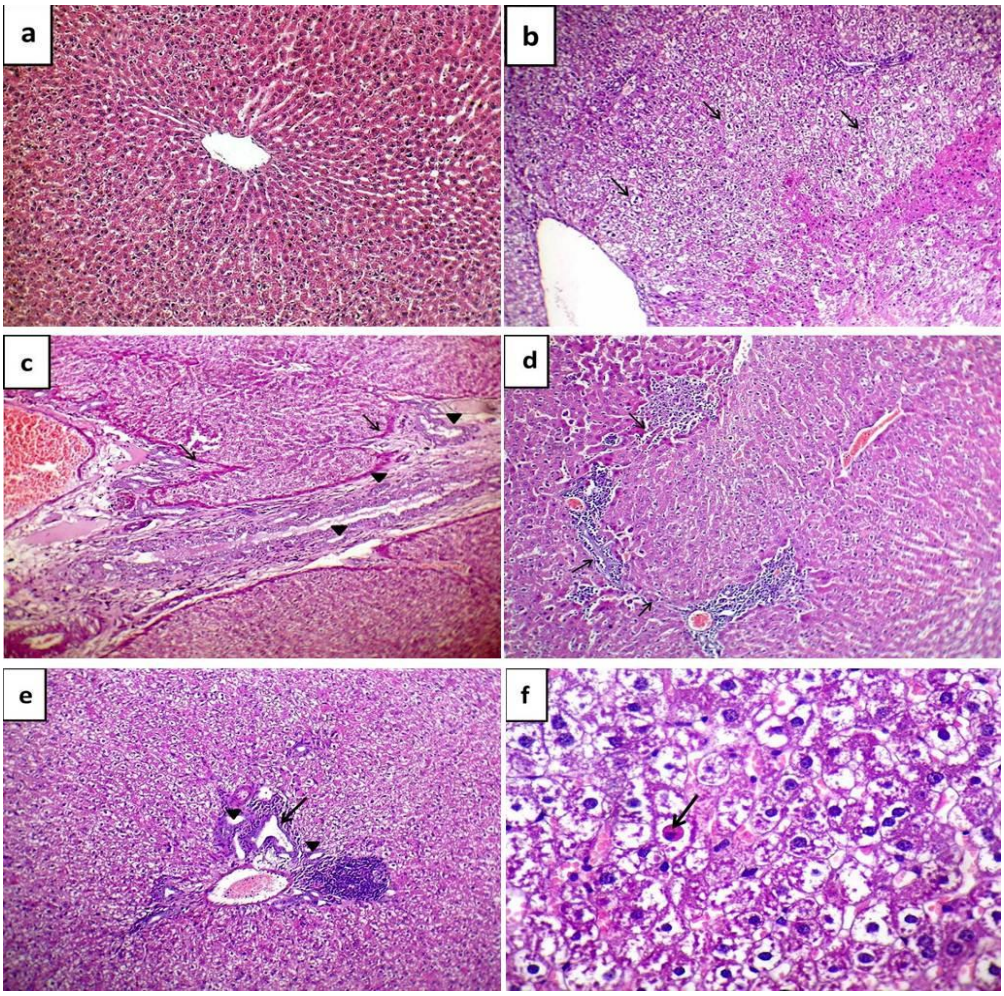

Fig. 1. Liver of control (a) and TAA-treated rats (b-f) stained by H&E (x 100). After four weeks of experiment: (a) normal hepatic histoarchitecture. (b) Diffuse cytoplasmic vacuolation of the hepatocytes mainly of hydropic type (arrows). (c) Papillary hyperplasia (arrow) and formation of newly formed bile ductules (arrowheads). At ten weeks of the experiment (d) Extended hepatic necrosis to involve the neighboring lobule forming a thin necrotic tract (bridging necrosis) (arrows). (e) Marked thickening of portal area with mononuclear infiltrates, newly formed bile ductules (arrowheads) and mild fibroplasia (arrow) that extended giving the nodular appearance. (f) Apoptotic body formation (arrow) (x400).

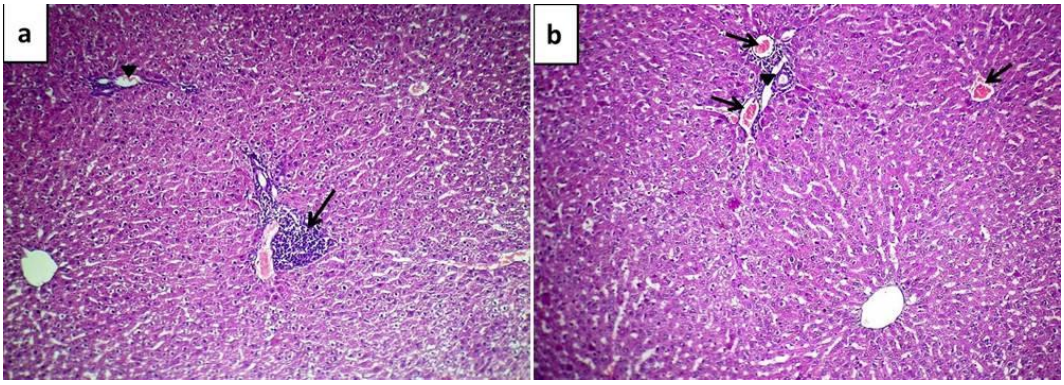

Fig. 2. Liver of a rat treated with TAA+ALA, stained by H&E (x100): (a) At four weeks of the experiment, showed moderate thickening of portal area with mononuclear cells infiltration mainly lymphocytes (arrow) and other one appeared normal (arrowhead). (b) At ten weeks of the experiment, showed mild congestion (arrowhead) and mononuclear cell infiltrates in the portal areas (arrows).

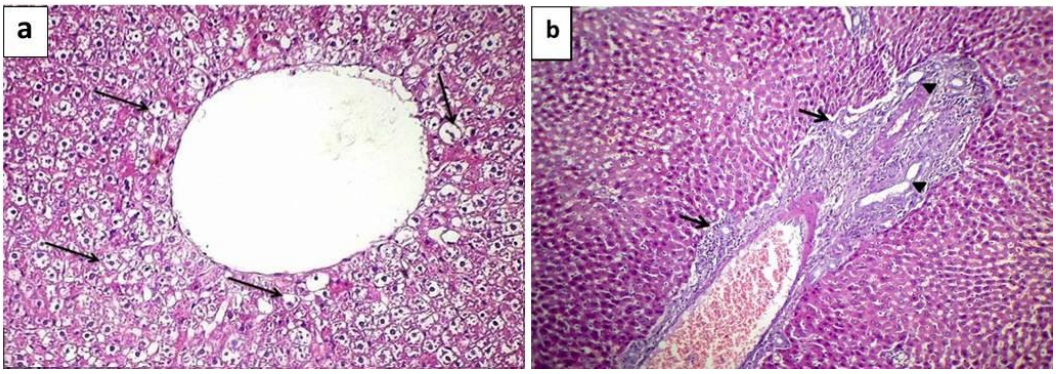

Fig. 3. Liver of a rat treated with TAA+SIL, stained by H&E. (a) after four weeks of the experiment, Diffuse hepatocytic vacuolation of the hydropic type (arrows), (x250)**.** (b) after ten weeks of the experiment, Extended portal area with dilated portal vein, mononuclear inflammatory cells, biliary epithelial hyperplasia (arrow) and mild fibroplasia (arrowheads). (x100).

4. DISCUSSION

Thioacetamide (TAA) causes various degrees of liver damage including nodular cirrhosis, liver cell proliferation, formation of pseudolobules and parenchymal cell necrosis (Mitra et al., 1998). TAA induced hepatic necrosis by generating free radicals (Mohammed et al., 2009). The hepatotoxicity of TAA results from its hepatic biotransformation by both flavin-containing mono-oxygenase (FMO) (Malvaldi et al., 1984) and cytochrome P450 (Lee et al., 2003) reduce dioxygen to superoxide anion, which is then catalyzed in rats to form hydrogen peroxide (Ekstrom et al., 1989), resulted in oxidative damage associated liver injury. This was demonstrated by the elevation of hepatic MDA "an index of lipid peroxidation" and decrease hepatic GSH and GST contents in TAA-treated group. In the present study, administration of TAA to normal rats showed significant increase in hepatic MDA concentration, with a significant decrease in GSH content and activity of GST in liver homogenate as compared to control rats (Moustafa et al. 2015). In the current study, administration of TAA triggered a significant elevation of serum liver enzymes (ALT, AST, ALP and GGT), as well as, total and direct bilirubin, with a time dependent increase at four and ten weeks post-treatment comparing to control group. The increase in serum liver enzymes activity of TAA- treated rats might be attributing to increased permeability of plasma membrane or cellular necrosis induced by TAA toxic metabolite free radicals resulting in leakage of the enzymes to the blood stream (Alshawsh et al., 2011). Serum AST and ALT activities are used as very sensitive markers for necrotic lesions within the liver due to their easy liberation from the hepatocyte cytoplasm into the blood stream (Kirchain and Gill, 1997) as a result of membrane lipid peroxidation (Wendel et al., 1979). Activity of serum ALP can use as a marker for the assessment of hepatobiliary damage in rats (Boone et al., 2005). Moreover, GGT is a membrane-bound enzyme, present mainly in the canalicular ducts; whose serum activity is increased in cholestatic liver disease resulted from impaired bile flow and necrosis of biliary epithelial (Hall, 2001). The

elevation in the serum total bilirubin concentration may occur as a result for cholestasis with subsequent impairment of intrahepatic or extrahepatic bile flow resulted in bile retention. Moreover, the accelerated destruction of erythrocyte or alteration in bilirubin metabolism may lead to increased production of serum total bilirubin (Hoffmann et al., 1999 and Meyer and Harvey, 2004). Also, our data revealed a significant increase in the concentration of serum direct and total bilirubin in TAA-treated group. This may be attributed to impairment of bilirubin metabolism and excretion (Michelli, 2011). Similar findings were reported by Ali et al. (2016) and Osman (2016). Liver has a vital role in synthesis of most serum proteins, particularly fibrinogen, albumin and other coagulation factors and most of the alpha and beta globulins (Thapa and Walia, 2007). The significant hypoproteinemia and hypoalbuminemia in TAA-treated rats in the current work could be related to the induction of ubiquitin-associated protein degradation (Andersen et al., 1981). As well, the necrotic cells become unable to metabolize protein or synthesize albumin leading to a decrease in total proteins and albumin (Ismail et al., 2009). The obtained biochemical results of serum liver enzymes and bilirubin concentrations were confirmed by our histopathological findings in TAA-intoxicated rats that were time dependent. The histopathological alterations were confined to both hepatic cells and portal areas that showed moderate to great thickening by mononuclear cells infiltration and biliary epithelial hyperplasia and then progressed by the time giving more severe lesions at ten weeks post to involve bridging necrosis as well as markedly thickened and extended portal area giving the nodular appearance. Similar results were documented by Abo El-Magd et al. (2015) and Osman (2016). Moreover, TAA intoxication disturbs lipid metabolism as our data revealed a significant elevation in serum TG and TC with a significant reduction in HDL in TAA-treated rats in addition to hepatocytic vacuolation of fatty type that were found at the ten weeks of the treatment (Mustafa et al., 2013). Supplementation of ALA and SIL have a hepatoprotective properties against TAA-induced hepatic damage that reflected by a significant decrease in the activities of serum liver enzymes (ALT, AST, ALP, and GGT), total and direct bilirubin, as well as, TC and TG levels. While serum total proteins, albumin and HDL levels showed a significant increase in in rats treated with TAA+ALA and/or TAA+ SIL comparatively to TAA-intoxicated rats in a time dependent manner. Also, our histopathological findings showed mild lesion in mixed treated groups as compared to TAA-intoxicated group. The ability of ALA to ameliorate the TAA-induced elevation in serum liver enzymes, bilirubin, might be correlated to both its radical scavenging properties, indirect effect as a regulator of antioxidative systems (Abdel-Daim et al., 2015 and El-Feki et al., 2016). The beneficial hypolipidemic effects of ALA were in accordance with the results obtained by Mythili et al. (2006), who found that ALA supplementation was effective against cyclophosphamide-induced dyslipidemia. The mechanism by which ALA is able to reduce total cholesterol is not clear, but probably via modulation of lipoprotein lipase activity or cholesterol metabolism by the liver (Tomonaga et al., 2005). Moreover, ALA supplementation revealed a significant decrease in MDA with a significant increase of GSH and GST in liver of rats treated with TAA+ALA in a time dependent manner compared to TAA-treated rats. Wollin and Jones (2003) reported that ALA and its reduced form (DHLA) are potent antioxidants, not only act directly (by radical quenching such as H2O2, OH, singlet oxygen and metal chelation) but also indirectly, through the regenerating of other endogenous antioxidants such as ascorbic acid, vitamin E, and glutathione. Moreover, ALA can restore the inhibited activities of enzymatic antioxidants, as well as, the decreased levels of non-enzymatic antioxidants (Abdel-zaher et al., 2008). Also, ALA provided protection to the hepatic histoarchitectue and ameliorated the histopathological lesions induced by TAA (Çakır et al. 2015). While the hepatoprotective role of SIL against TAA-induced elevated serum enzymes could be attributed to its antioxidant effect and its ability to act as a free radical scavenger, thereby protecting membrane permeability (Elhaak et al., 2015). The improvement of liver tissue cells due to SIL- treatment was recorded previously by Hamza and Al-Harbi (2015) and Elhaak et al. (2015). The ability of SIL to protect against TAA-induced alterations in serum lipids might be resulted from their ability to decrease serum TC and TG levels, in addition to slowing the process of lipid peroxidation by improving antioxidant enzyme activity. The ability of SIL to ameliorate TAA-induced oxidative stress in liver may attributed to enhancing DNA polymerase, stabilizing cell membrane and scavenging free radicals (El-Shafeey et al., 2012 and Hermenean et al., 2015). Treatment with ALA and SIL ameliorated TAA-induced oxidative damage, alterations in the liver function tests and liver histopathology. However, ALA demonstrated better hepatic protection against TAA-induced liver

damage as compared to SIL. Finally, it is concluded that ALA has more powerful antioxidant properties than SIL.

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