Antioxidant effect of Flaxseed against liver Cirrhosis induced in Thioacetamide intoxicated rats

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Abstract:

Background/Aim: There has been a current upsurge in the medical implications of free radicals and related species during the past several decades. These chemical species are integral components produced during normal biochemical and physiological processes but leads to oxidative stress when produce in excess and causes potential damage to cells. A wide range of non-enzymatic and enzymatic antioxidant defenses exists to counteract the damaging effects of free radicals. There exist epidemiological evidences correlating higher intake of antioxidant rich foodstuffs with greater free radical neutralizing potential to lower incidence of several human morbidities or mortalities.

Material and Methods: Liver cirrhosis model was induced by TAA, hepatobiochemical and antioxidant parameters were assayed.

Results: Flaxseed induced enhancement in liver function test, antioxidant status and lipid profile.

Conclusion: Flaxseed can be used as hepatoprotective against liver cirrhosis induced with TAA as compared to reference drug Silymarin group.

Keyword: liver Cirrhosis, Thioacetamide, Flax seed, Silymarin, Antioxidant.

Introduction

The liver plays a crucial role in the metabolic elimination of most drugs and other foreign compounds, thus making it an important target for toxicity. Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions [1]. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in Western countries [2].

Thioacetamide (TAA) was originally used as a fungicide to preserve agricultural citrus products, but later it was determined that it was a potent hepatotoxin and carcinogen because its thono-sulfur-containing compound endowed with liver-damaging and carcinogenic activities [3]. On a prolonged exposure, TAA leads to the formation of hyperplastic nodules, cell adenomas, hepatic carcinomas, and cirrhosis. TAA-induced cirrhosis in rats has been shown to be a suitable experimental model of this disease with etiology and pathology comparable to the one seen in humans [4].

In recent years, flaxseed is gaining importance in diet supplements, as they possess nutraceutical properties. Flaxseed is one of the oldest domesticated crops and it is being increasingly used in the human diet because of its potential health benefits, particularly for cardiovascular protection [5].

Flaxseed is the richest natural source of plant lignans, with secoisolariciresinoldiglucoside (SDG) being the principal lignan compound, chemically 2, 3-bis [4-hydroxy-3-methoxyphenyl]methyl]-1, 4-butane diglucoside. The concentrations of SDG in flaxseed vary with different cultivars. SDG concentrations in twenty-seven flaxseed species ranged from 1.19 to 2.59% for SDG and 0.22 to 0.5% (w/w) for its diastereoisomer [6]. Flaxseed commonly contains 34–45.6% total fat, and a-
linolenic acid alone represents 45–60% of the total fatty acid content in flax oil. Although α-linolenic acid is an ω-3 fatty acid, dietary α-linolenic acid did not show cholesterol-lowering effects in several clinical trials [7].

Silymarin is a purified extract obtained from the seeds of the plant Silybum marianum and used widely as a supportive therapy for liver disorders such as cirrhosis, hepatitis, and fatty acid infiltration due to alcohol and toxic chemicals [8]. In this study, the hepatoprotective function of the hull flaxseed was further evaluated and its efficacy as a therapeutic agent was experimentally tested on a rat model of liver cirrhosis induced chemically by TAA administration.

**Material and methods**

**Animals**
A total of 70 Sprague Dawley adult male albino rats weighing between 180-230 gm were randomly divided into 7 equal groups. They were individually housed in metallic cages under constant healthy environmental conditions. Water and diet were provided ad libitum.

**Preparation of Flaxseed**
Flaxseed (L. usitatissimum) was purchased from Agricultural Research Center. As a hull fraction of flaxseed was ground and mixed with basal diet treatment.

**Preparation of Thioacetamide**
TAA (Sigma-Aldrich) was prepared freshly by dissolving in sterile distilled water and stirred well until all crystals were dissolved. Then, 200 mg/kg body weight was administered intraperitoneally (ip) to the rats twice weekly for 4 consecutive weeks. The injection protocol above was according to the recommendation of Bruck [9]. Constant exposure of rats with this amount of TAA induces changes in its liver pathology from both biochemical and morphological aspects comparable to that of human liver cirrhosis [10] and therefore, used very often as a preferred model in experimental Studies of this disease.

**Preparation of Silymarin**
Silymarin (70% silibin, 16.5% silydianin, and silychristin) was purchased from Sedico Pharmaceutical Company (Egypt). Silymarin which is a reference drug and (5mL/kg body weight) was dissolved in saline for oral administration to rats in a dose of 20mg/kg body weight according to the recommendation of Rastogi [11].

**Animal diets**
The stock basal diet was prepared according to the recommended proportions given by reeves [12], as shown in table (1).

**Experimental design**
Seventy male rats were randomly divided into seven groups, each of which with ten rats: Group 1 (control group) rats were received basal diet for five weeks and intraperitoneally (ip) injected with saline solution. Group 2 and group 3 (Flaxseed control groups) rats were served as a flaxseed control group and they were received basal diet supplemented with flaxseed (5% and 10%) respectively for five weeks. Group 4 (cirrhotic group) was received the basal diet for five weeks after the first week rats were intraperitoneally injected with thioacetamide at a dose of 200 mg/kg body weight twice weekly for 4 consecutive weeks. Group 5 (reference drug group) was received the basal diet for five weeks with oral administration of silymarin at a dose of 20 mg/kg body weight, after the first week rats were received the same course of thioacetamide as the cirrhotic group. Group 6 (5% Flaxseed group) was received flaxseed supplemented diet (5%) for five weeks, after the first week they were received the same course of thioacetamide as the cirrhotic group. Group 7 (10% Flaxseed group) was received flaxseed supplemented diet (10%) for five weeks, after the first week they were received the same course of TAA. Body weights of rats were recorded at three days interval to monitor body weight change and to determine the doses for induction of fibrosis and silymarin dose. At the end of five weeks (experimental period), the animals were fasted 12 hrs, then they were anaesthetized with diethyl ether and blood was collected.

**Food intake and body weight variation.**
Food intake was controlled, being the difference between the offered and leftover food. Body weight of each animal was evaluated 2 times a week, throughout the experiment, which lasted 35 days table.

**Antioxidant Activity**

Hepatic tissues from all livers were sampled from the same site, but away from the portal system. One gram of the sampled tissue was placed in 10mL (10% w/v) of PBS (phosphate buffer solution with pH 7.4), then homogenized and centrifuged at 4000 rpm for 10min at −4°C. The supernatant was kept in a −80°C freezer and it was used to determine the Glutathione-S-transferase (GST), Reduced glutathione (GSH), Malondialdehyde (MDA) and superoxide dismutases (SOD).

**Hepatic Biochemical Parameters**

Blood of each rat was collected and serum was separated for analysis to determine the liver function enzymes such as alanine aminotransferase (ALAT), aspartate amino-transferase (ASAT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and arginase activity.

**Serum Lipid profile**

Collected serum was used to determine levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TAG) and very low-density lipoprotein cholesterol (VLDL-C) were determined.

**Statistical Analysis**

Statistical analysis was evaluated by using one-way analysis of variance (ANOVA) using QI macros 2010. Data were expressed as the mean ± SD; a probability value less than 0.05 was considered significant.

**Results**

**Body weight change, hepatosomatic, Spleenosomatic, nephrosomatic, cardiosomatic, gonadosomatic indices and food intake**

The hepatotoxic weight change and food intake were extremely significantly decreased, but their indices of hepatosomatic, Spleenosomatic, nephrosomatic, cardiosomatic and gonadosomatic were significantly more compared with the control rats. Rats subjected to the treatment with silymarin exhibited extremely significantly loss in the body weight change but the amounts were not as much as those in the cirrhotic group. Rats subjected to the treatment with 5% flaxseed exhibited extremely significantly elevation in the indices of hepatosomatic, Spleenosomatic, nephrosomatic, cardiosomatic and gonadosomatic (Figure 1, Figure 2 and Figure 3).

**Hepatic Biochemical Parameters**

There were an extremely significant increased in the serum ALAT, ALAT, ASAT and a significant increase in LDH and arginase were detected in the hepatotoxic rats compared to those in the control group. Silymarin therapy was effectively restored the activities of ALAT, LDH and arginase but showed extremely significant increase in ALP activity however the amounts were not as much as those in the cirrhotic group and showed extremely significant decrease in ASAT activity. Cirrhotic groups treated with 5% and 10% flaxseed produced activities similar to their respective 5% and 10% flaxseed control groups except in case of 10% flaxseed group which showed an extremely significant increase in ASAT and LDH activity as compared to cirrhotic 10% flaxseed group (Figure 4).

**Antioxidant activity**

Figure 5 lists the activities of enzymes involved in the hepatic antioxidant defense system. It showed that MDA and SOD enzyme levels were extremely increased in cirrhotic group while GSH and GST level were extremely decreased when compared with the control group. GST and GSH enzymes were restored their level insilymarin group while showed a significant increase in MDA and SOD levels as compared to control group.
activity but the amounts were not as much as those in the cirrhotic group. The cirrhotic rats treated with flaxseed showed increases in antioxidant level as compared to control group.

**Lipid profile**

As shown in figure (6). Serum total cholesterol level was significantly decreased in 5% flaxseed control group and 10% flaxseed control by 16.8% and 35.4% respectively as compared to control group. High density lipoprotein-cholesterol (HDL-cholesterol) showed a significant decrease in liver cirrhotic rats.

**Discussion**

Liver cirrhosis is a major disease associated with various pathological processes including progressive fibrosis, portal hypertension and carcinoma [13]. Free radical generation, mitochondrial dysfunction and depletion of antioxidants lead to the progression of fibrosis and cirrhosis [14]. Thioacetamide is an organic solvent with thiono-sulfur components which have been used widely to induce liver cirrhosis [15–19]. In the present study, the prolonged administration of TAA to the rats caused visual and quantifiable responses, which were recognizable by the alterations in the body and organ weights. TAA contributes to the development of cirrhosis through multiple mechanisms of action [20] like the oxidation of its metabolic products [21, 22], oxidative stress [23, 24], and decreased antioxidant defenses and lipid peroxidation. During the five-week-long study, the hepatotoxic rats lacked in gaining body weight as compared with the controls (figure 1). Using the same experimental model of cirrhosis, the previous studies reported the same and attributed this outcome to the lower levels of nutrient absorption, energy utilization, and metabolic efficiency as the major factors affecting the inability of the rats to gain weight after being exposed to TAA [25]. Factorizing the reduced body weight into the calculation yielded significantly high ratios of liver, spleen, kidney, testis, and heart-to-body weight. The hepatocyte proliferation is a critical determinant for the survival of liver from an injury [24]. Based on this, the upregulation of the hepatocyte activity in response to the exposure to TAA toxicity is likely to be the cause of the recorded increase in the organ weights (figure 2).

In the cirrhotic group, we detected elevated levels of serum ALAT, ASAT, ALP, LDH, and Arginase concentrations (figure 3), which were typically measured for assessing the liver function. The increase in serum enzymatic activities is related to hepatic parenchymal damage since ALAT is released from mitochondrial and cytosolic localization from membranal sites, and cellular rupture allows the enzyme to escape into the blood [26]. The raised serum liver enzymes such as ALAT, ASAT, ALP, LDH and Arginase intoxicated rats compared to normal indicates necrosis of hepatocytes that results in the leakage of transaminase and the elevation of serum ALAT, ASAT, ALP, LDH and Arginase.

On the other hand, TAA group exhibited higher values of cellular lipid peroxidation which measured through the malonaldehyde level (MDA) than did the control livers [27].

The toxicity of TAA results from its bioactivation in the liver to active metabolites, causing the production of ROS responsible for oxidative stress [28, 29]. These events are followed by glutathione depletion, a reduction in SH-thiol groups and oxidation of cell macromolecules, including lipids [30, 31]. Our results are in agreement with these findings (figure 4). Glutathione plays a key role in detoxification of ROS and reactive electrophilic compounds [32, 33]. In tissues, glutathione occurs in a reduced (GSH) and oxidized form (GSSG). More than 99% of the total glutathione occurs as GSH (34, 35). In our experiments control hepatocytes contained about 85% of the total glutathione in the form of GSH. This could be explained by the fact that, in vitro experiments, cells are artificially exposed to hyperoxia, thus increasing oxidative stress [36].
Silymarin is a well-established plant-based formulation and a clinical drug with proven capacity to guard liver from harmful hepatotoxins. Such pharmacological power was attributed to silymarin’s inherent constituents with antioxidant, anti-inflammatory, and diuretic properties, as in other medicinal plants in nature [37]. In addition, silymarin has the capacity as antilipid peroxidation and induced detoxification system, protector of cell against employed glutathione, reducer of leukotiene formation from unsaturated free acid, enhancer of protein synthesis, stabilizer of mast cells and regulator of immune functions. It inhibits cytoP450 detoxification system and prevents metabolism of toxic compound such as TAA [38].

In our study, we reconfirmed that silymarin played substantial role against the progression of cirrhosis induced by TAA in rats. It significantly reduced the liver pathology indicated by the declined levels of ALAT, ASAT, ALP as compared to the hepatotoxic rats. The readings for these biochemicals were the best achievable values and therefore set the baseline for comparisons of therapeutic improvements attained with the flaxseed [39].

Flaxseed lignan is a precursor of the mammalian lignans, enterodiol [ED; 2,3-bis[(3-hydroxy-phenyl)methyl]-1,4-Butanediol; MW 302] and enterolactone [EL; trans-dihydro-3,4-bis[(3-hydroxyphenyl)methyl]-2(3H)-Furanone; MW 298] via the activity of colonic facultative aerobes (Clostridia sp.). During this conversion, SDG first undergoes hydrolysis to yield the aglycone plant lignan secoisolariciresinol (SECO); [R-(R'R'R*0,R*)]-2,3-bis[(4-hydroxy-3-methoxy phenyl)methyl]-1,4-Butanediol; MW 362], which is then dehydroxylated and demethylated to yield ED; ED can then be oxidized to EL. The mammalian lignans differ from the plant precursors due to the presence of phenolic hydroxyl group moieties only in the meta position on the aromatic rings, compared to the 3-methoxy-4-hydroxyl substituents on the A and B rings of the parent molecules SDG and SECO [40].

The flaxseed lignan and its mammalian metabolites have been reported to exert protective effects against diet-related chronic diseases through a variety of mechanisms including phytoestrogenic and antioxidant effects from in vitro and in vivo studies [41, 45].

The flax-induced increase in the liver enzyme GSH, which we propose to play a role in flax-induced hepatoprotection, is due to the lignan, likely SDG, and not the oil component of flaxseed [46].

### Conclusion
All the observations made and measurements collected in this study provided preliminary evidence that the progression of the liver cirrhosis induced by TAA in rats can be intervened using the flaxseed. Specifically, this natural seed has power to protect the liver by preventing the actions of the harmful events associated with the TAA toxicity from taking place. By preservation of GSH levels in the normal and injured. The effects are comparable to those of silymarin and the capability of the flaxseed to preserve the liver’s status quo of property, structure, and function against toxic exposure is encouraging and warrants further studies exploring the significance of its pharmacologic potential in treating the liver cirrhosis by mapping the molecular pathways of action.

### References
3. Al-Bader A, Mathew T, Abul H, Al-Mosawi M, Dashti H, Kumar D and Singal P


Table (1): AIN-93M diet formulated for maintenance of adult rodent

<table>
<thead>
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Fig (1): Mean of food intake of intake in experimental groups.

Fig (2): Mean of body weight change in the experimental groups.
Fig (3): Mean of organs indices in the experimental groups.
Fig (4): Mean of serum liver enzyme activity and Arginase activity in the experimental groups.
Fig (5): Mean of hepatocellular GST, GSH, MDA and SOD in the experimental groups.
Fig (6): Mean of serum lipid profile in the experimental group