

Therapeutic Effect of *Silybum marianum* Plant Extract on Tamoxifen-Induced Fatty Liver in Rats

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Background: Tamoxifen (TMX) is a synthetic drug that is widely used for treatment of breast cancer. Despite the beneficial effects of TMX, the use of this drug has several side effects such as the development of Non-Alcoholic Fatty Liver Disease (NAFLD) in the patients. *Silybum marianum* is the most researched plant in treatment of liver disease.

Objectives: In this study the effect of *Silybum marianum* Extract (SME) on histological and biochemical parameters in TMX-treated rats was investigated.

Materials and Methods: Adult female Wistar rats were divided to four groups; 1) normal control untreated rats, 2) SME-treated rats, that received only SME in a dose of 1.6 g/kg body weight/day, oral gavage for 14 days, 3) TMX-treated rats, that received TMX in a dose of 1 mg/kg body weight/day, subcutaneously, for seven days, 4) TMX-SME-treated rats, that received TMX in a dose of 1 mg/kg body weight/day, subcutaneously, for seven days and then SME by oral gavage for 14 days. The groups were compared in terms of serum glucose, triglyceride, cholesterol, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), High-Density Lipoprotein-Cholesterol (HDL-C), Low-Density Lipoprotein-Cholesterol (LDL-C), total protein and liver triglyceride amount, and histological findings.

Results: Concentrations of HDL-C and protein decreased and activity of ALT increased in TMX group compared with the control group. The SME increased protein concentration and decreased ALT activity in TMX-SME group compared with the TMX group. Histopathological examination and triglyceride assay in liver tissues showed lipid accumulation in the TMX-treated rats and amelioration of lipid accumulation in the liver of TMX-SME-treated rats.

Conclusions: Intake of water extract of *Silybum marianum* is a useful treatment for liver function in drug-induced fatty liver.

Keywords: Fatty Liver; Tamoxifen; *Silybum marianum*

1. Background

Tamoxifen (TMX), 2-[4-[(Z)-1,2-diphenylbut-1-enyl phenoxy]-N,N-dimethylethanamine, is a synthetic non-steroidal anti-estrogen drug that is widely used for treatment of breast cancer (1). It is also used for infertility treatment due to its stimulatory effect on the secretion of pituitary gonadotropin hormones (2, 3). Despite the beneficial effects of TMX, the use of this drug has several side effects in patients with breast cancer (4). Hepatic side effects of TMX include elevation in liver function tests, jaundice, hepatitis, steatohepatitis, cholestasis and massive hepatic necrosis (5). Non-Alcoholic Fatty Liver Disease (NAFLD) is a well-known adverse effect of TMX (4). This disease is the accumulation of fat in the liver in patients without a history of alcohol abuse. Non-Alcoholic Fatty Liver Disease is classified into simple steatosis and Non-Alcoholic Steatohepatitis (NASH). Simple steatosis is a benign condition that can be reversed by some drugs, whereas NASH is an irreversible form of the disease, in which not only steatosis but also hepatocellular inflammation injury are present and can often cause progress to fibrosis, cirrhosis and Hepatocellular Carcinoma (HCC) (6). Herbal medicines derived from plant extracts due to their

low cost, availability, and fewer side effects, are increasingly being used for treatment of a wide variety of clinical diseases (7, 8). Among various plants, *Silybum marianum* (milk thistle) is the most researched plant for the treatment of liver diseases from ancient times until now (9, 10). Several studies showed that the extract of this plant protects the liver against chemical and environmental toxins (11-14).

2. Objectives

In the current study we investigated the effect of *Silybum marianum* on histological and biochemical parameters in TMX-treated rats.

3. Materials and Methods

3.1. Chemicals

Tamoxifen was purchased from Sigma, USA. Biochemical assay kits were purchased from Pars Azmoon, Iran. The other chemicals used were of analytical grade.

3.2. Preparations of Aqueous *Silybum Marianum* Extract (SME)

Silybum marianum extract was prepared as described previously (11). Briefly, 5 g seeds of this plant were dissolved in 60 mL of boiling distilled water and left to brew for 10 to 15 minutes. After filtration, 4 mL of this solution was given to the rats by gavage, daily.

3.3. Induction of Fatty Liver in Rats by Tamoxifen

Tamoxifen dissolved at a concentration of 0.2 mg/mL in sesame oil, containing 1% benzyl alcohol, was subcutaneously injected into the rats, 1 mg/kg body weight/day for seven days (15).

3.4. Animals and Treatment

Adult female Wistar rats weighing 190 - 210 g were purchased from the Pasteur Institute of Iran. They were kept in metal wire cages in a room with 12-hour light-dark cycles, at a constant temperature of $25 \pm 2^\circ\text{C}$ and free access to food and water. The animals were acclimatized for at least five days under these conditions before the start of the experiments. The rats were divided to four groups of six animals. 1) Normal control untreated rats. 2) SME-treated rats; this group received only SME, at a dose of 1.6 g/kg body weight/day, by oral gavage for 14 days. 3) TMX-treated rats, this group received TMX at a dose of 1 mg/kg body weight/day, subcutaneously, for seven days. 4) TMX-SME-treated rats, this group received TMX at a dose of 1 mg/kg body weight/day, subcutaneously, for seven days and then SME by oral gavage for 14 days.

3.5. Biochemical Assay

Biochemical parameters such as level of glucose, triglycerides, cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-

C), and total protein in sera were determined using Pars Azmoon diagnostic kits. For evaluation of liver function, activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) in sera were determined by Pars Azmoon diagnostic kits. To distinguish triglycerides accumulation in liver tissues, triglyceride concentration was quantified by a colorimetric assay after extraction by the method of Folch et al. (16). Tissues were homogenized in chloroform/methanol (2/1) to a final volume of 20 times the volume of the tissue sample (1 g in 20 mL of solvent mixture).

3.6. Liver Histological Analysis

The liver tissues of different groups were quickly removed at the end of treatment, cut into small pieces, soaked, and fixed in 10% aqueous formalin to paraffin embedment. Staining of paraffin sections with hematoxylin and eosin (H and E) was done for histological evaluation.

3.7. Statistical Analysis

Statistical analysis was performed with the SPSS software Version 16 (SPSS, Chicago, IL, USA) and Analysis of Variance (ANOVA) was used to compare means in different groups. A probability (P) value of less than 0.05 was considered significant in all statistical analyses. All experiments were performed independently at least three times. Data are expressed as Means \pm Standard Deviation (SD).

4. Results

Table 1 shows the serum levels of glucose, protein, triglycerides, cholesterol, HDL-C and LDL-C in control and experimental groups of rats. The levels of glucose, triglycerides, cholesterol, and LDL-C did not change significantly, whereas protein and HDL-C concentration were significantly decreased ($P < 0.05$) in the TMX group compared with the control group. Oral administration of SME increased the protein

Table 1. Biochemical Parameters in the Serum of Rats ^{a, b}

Groups	Glucose, mg/dL	Protein, g/dL	Triglycerides, mg/dL	Cholesterol, mg/dL	HDL-C, mg/dL	LDL-C, mg/dL
Control	175.6 \pm 17.5	8.1 \pm 0.7	152.2 \pm 34.5	63.7 \pm 2.8	41.6 \pm 11	2.6 \pm 1.1
SME	199.7 \pm 14.9	8.1 \pm 0.6	126.5 \pm 45.8	68.2 \pm 7.3	46.5 \pm 5.1	2.0 \pm 0.01
TMX	161.6 \pm 13.8	7 \pm 0.2 ^c	164.8 \pm 30.3	47.3 \pm 14.3	24 \pm 9.4 ^c	1.7 \pm 0.8
TMX + SME	182.6 \pm 26.2	7.6 \pm 0.3 ^d	182.1 \pm 58.3	59.3 \pm 8	32.6 \pm 10.3	1.4 \pm 0.5

^a Abbreviations: HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SME, *Silybum marianum* extract; TMX, tamoxifen.

^b Data are presented as Mean \pm SD.

^c $P < 0.05$, significantly different from the control group.

^d $P < 0.05$, significantly different from the TMX group.

Table 2. Liver Enzymes Activity in the Serum of Rats ^{a, b}

Groups	ALT	AST	ALP
Control	56 \pm 5.8	91.5 \pm 8.7	323 \pm 52.6
SME	54.5 \pm 7.4	98.5 \pm 12.1	310 \pm 85.3
TMX	77 \pm 15.6 ^c	118 \pm 5	386 \pm 106.1
TMX+SME	60.3 \pm 5.8 ^d	101.6 \pm 9.3	336.7 \pm 56

^a Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; SME, *Silybum marianum* extract; TMX, tamoxifen.

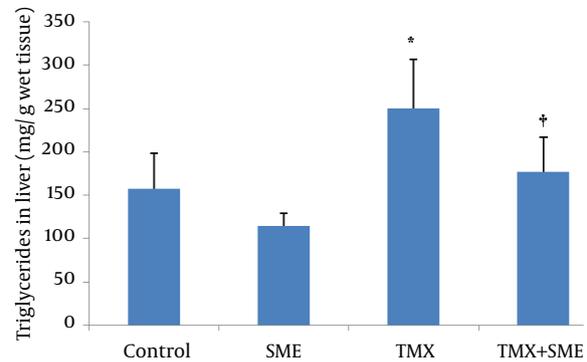
^b Data are presented as Mean \pm SD, unit is U/L.

^c $P < 0.05$, significantly different from the control group.

^d $P < 0.05$, significantly different from the TMX group.

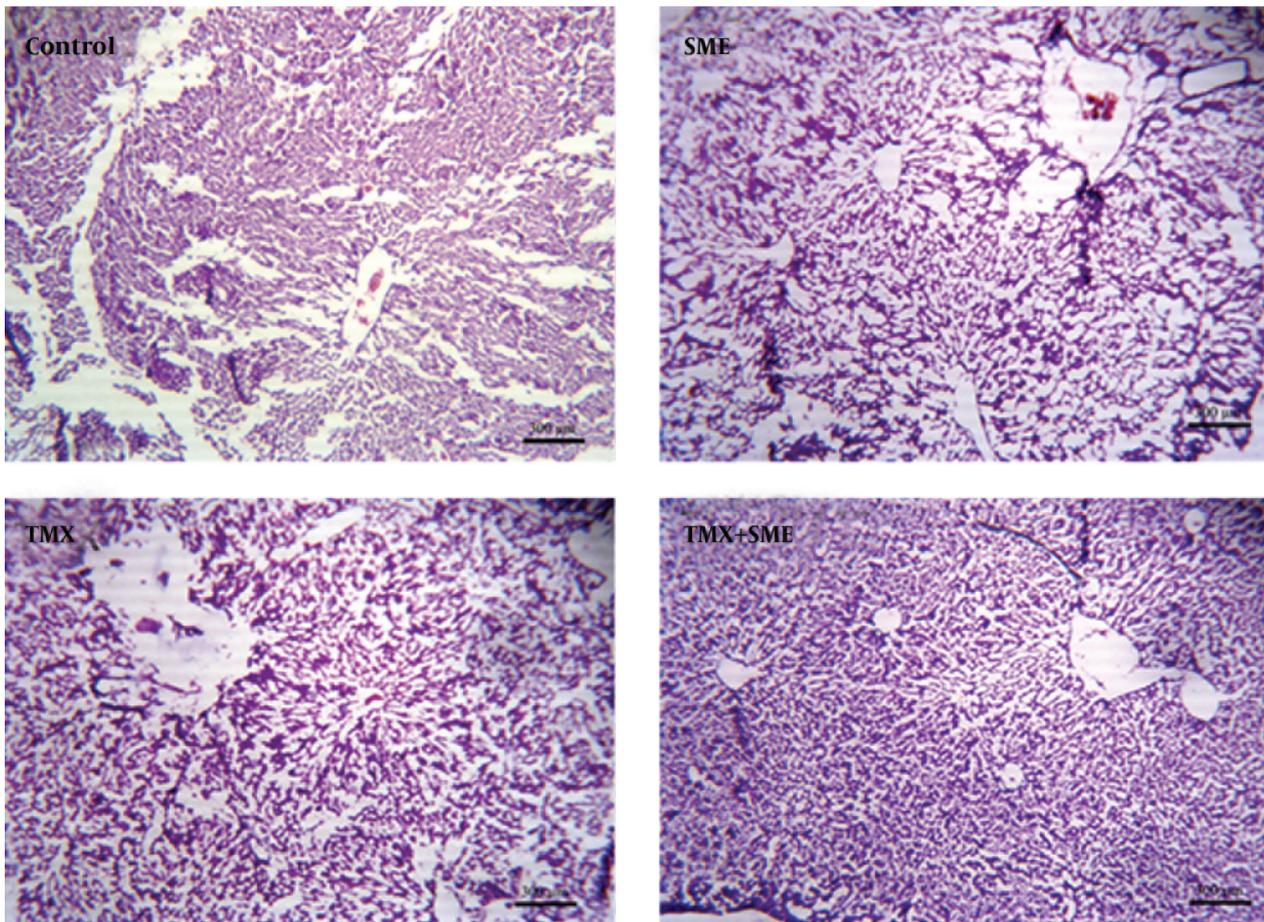
level significantly ($P < 0.05$) and the HDL-C concentration non-significantly, in TMX+SME group compared with the TMX group. Liver function test results are shown in Table 2. The activity of ALT was elevated significantly ($P < 0.05$) in TMX-treated rats in comparison with the control group. Oral administration of SME in the TMX-SME group decreased this enzyme activity significantly ($P < 0.05$) close to normal levels. Activities of AST and ALP followed the same pattern, although they were not significant. Figure 1 depicts the level of triglycerides in the liver tissues of control and experimental animals. A significant increase ($P < 0.05$) in triglyceride level was observed in TMX-treated rats, which changed to normal values after treatment with SME in the TMX-SME group. Histological results confirmed biochemical data showed that SME can improve fatty liver-induced by TMX (Figure 2).

Figure 1. Triglycerides Concentration in Liver Tissues



Abbreviations: SME, *Silybum marianum* extract; TMX, tamoxifen; values are means \pm SD; * $P < 0.05$, significantly different from the control group; † $P < 0.05$, significantly different from the TMX group.

Figure 2. Liver Histopathology (H & E, $\times 40$)



Abbreviations: SME, *Silybum marianum* extract; TMX, tamoxifen.

5. Discussion

Liver injury is one of the side effects of tamoxifen treatment in women with breast cancer. Tamoxifen is a toxic drug for liver tissue because of its higher affinity for hepatocytes compared with the other cells (17). Among various liver problems, NAFLD is the most common condition due to activation of fatty acid and triglyceride biosynthesis (18, 19). In these patients, liver enzymes activities in serum and liver sonography are abnormal (4). In this study tamoxifen was used in order to induce fatty liver in an animal model. As expected, liver enzyme activities in serum of TMX-treated rats elevated indicating liver injury. Although the increase of ALT serum levels was significant, it was not considerable for AST and ALP. High triglyceride concentration in liver tissues of TMX-treated rats proved lipid accumulation and fatty liver in these kinds of rats. Histopathological examination confirmed these biochemical results. Moreover, significant decrement of total protein concentration in serum of TMX-treated rats, was the reason for liver dysfunction. In similar studies with induction of fatty liver by drugs in animal models, different plant extracts were used for improvement of liver function (1, 7, 20). Madani et al. showed that *Silybum marianum* and *Cichorium intybus* had protective effects on rat liver cells treated by thioacetamide (10). Butt et al. demonstrated that *Cichorium intybus* had a hepatoprotective effect on paracetamol-induced liver damage in rats (21). Ozturk et al. showed that *Silybum marianum* had a preventive effect on CCl₄ induced liver damage (11). In this study *Silybum marianum* was used for treatment of lipid accumulation in fatty liver induction by tamoxifen. Because SME have a strong protection effect against multiple drugs (10, 22), applying SME in TMX-treated rats decreased liver enzymes activities close to normal levels. In addition, the rise of the serum total protein indicated that liver function was improved in the presence of SME. One of the most important problems caused by some drugs such as tamoxifen, is the induction of oxidative stress during their metabolisms in the liver (23). Since different plant extracts such as SME have antioxidant components, it might be that these compounds protect cells from damage caused by oxidative stress. On the other hand, the effect of SME on ameliorating of fatty liver is possibly due to inhibition of fatty acid synthesis or increasing of mitochondria and peroxisomal β -oxidation (18, 19). Data of this study indicated that SME has therapeutic effects on tamoxifen induced fatty liver. Further investigations are needed to determine the involved mechanisms.

Authors' Contributions

Design of the study, analysis of the data, and writing of the paper: Dr. Nasrin Ziamajidi. Experimental studies: Hamid Behrouj and Abolfazl Nasiri. Study cosupervision: Dr. Roghayeh Abbasalipourkabir. Histological examination: Dr. Sara Soleimani Asl.

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