Molecular and Biochemical Diagnosis (MBD)
Vol 1, No 3, 2014
Original Article

The effects of aqueous extract of chicory root on steatosis, lipid profile and liver damage enzyme markers in tamoxifen-treated rats

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Abstract

Background: Accumulation of triglycerides in the liver i.e. steatosis, is a well-known side-effect of tamoxifen administration to patients suffering from breast cancer. Cichoriumintybus (chicory) is a plant used as traditional medicine for curing liver disorders. In this study, the effects of extract prepared from chicory roots on tamoxifen-induced liver steatosis and related biochemical factors in animal model using rats has been investigated.

Methods: Female rats of Wistar strain were divided into four groups and treated as follows; 1-Control: received vehicle; 2- Chicory root-extract treated: rats were given by gavage the aqueous chicory root extract (1 g/kg body weight/day for 14 days).3- Tamoxifen-treated: rats received tamoxifen (1 mg/kg body weight/day, for 7 days). 4- Tamoxifen+chicory-group: animals received tamoxifen (1 mg/kg body weight/day for 7 days) followed by chicory extract given by gavage (1 g/kg body weight/day for 14 days). After treatment, blood was collected by cardiac puncher, plasma was separated and plasma levels of glucose, total protein, triglyceride, cholesterol,LDL-C, HDL-C and activities of ALT, AST and ALP were measured. Liver tissues were homogenized used for measuring tissue triglyceride and histological examinations.

Results: The data show that tamoxifen treatment caused a significant decrease in the level of serum cholesterol, HDL-C and total protein. However, serum ALT level was increased in tamoxifen-treated rats compared to controls. Increased serum ALT in tamoxifen-treated rats was recovered in rats treated with plant extract (tamoxifen+chicory-group). HDL-C and total protein levels were unaffected in rats fed chicory extracts. Tamaxifen-treated animals showed signs of liver steatosis as shown by histological examination and accumulation liver triglyceride. The steatosis markers such as accumulated triglyceride in liver was significantly reduced due to the plant extract treatments when compared to tamoxifen-group.

Conclusions: Dietary extract prepared from chicory roots is effective in modulation of tamoxifen-induced liver damage and steatosis.

Keywords: Cichoriumintybus, Steatosis, Tamoxifen, Rats, liver damage

Introduction

Steatosis of fatty liver is one of the chronic liver diseases which affect 10-24% of total world population. Initially it appears as benign steatosis, however the disease can progress to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.

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Non-alcoholic fatty liver disease (NAFLD) is the results of accumulation of lipids, especially triglycerides in the liver cells in patients without a history of alcohol abuse. Several factors are causing NAFLD, including obesity, metabolic syndrome, and consumption of some drugs (Paschos and Paletas, 2009).

Tamoxifen or Nolvadex is one of the drugs that may induce fatty liver (Murata et al., 2003; Liu et al., 2006). Tamoxifen, 2-[4-[(Z)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-dimethylethanamine, is a synthetic non-steroidal medication that is widely used for treatment of patients with estrogen receptor-positive breast cancer (El-Beshbishy, 2005). Due to its stimulatory effect on secretion of pituitary gonadotropin, it is also used in the treatment of infertility and bodybuilding supplements (Adashi et al., 1981; Motrich et al., 2007). Several studies have shown that approximately one third of breast cancer patients treated with tamoxifen develop hepatic steatosis (Nemoto et al., 2002).

*CichoriumIntybus*L., commonly known as chicory or Kasni (from the Asteraceae family), is a perennial plant with blue flowers that are grow in different regions in Iran. It is one of the oldest known plants used traditionally for treatment of liver diseases. The protective and curative effects of the root, leaves, and seeds of chicory have been investigated (Street et al., 2013). The major components of chicory roots are carbohydrates such as fructooligosaccharides and inulin and the minor components are polyphenols (Milala et al., 2009). Inulin has polyfructan structure which is water soluble and not hydrolyzed by digestive enzymes and belongs to a group of dietary fiber. Based on this it has been suggested that the water extract of chicory root possess hyperlipidemic properties (Kim and Shin 1998).

The objective of the current study was to examine the possible effects of aqueous extract of chicory root on fatty liver and related biochemical and histological markers in an experimental model of steatosis induced by tamoxifen.

Materials and Methods

Preparation of plant extract

Chicory roots were purchased from the medicinal herb store in Hamadan, Iran. A voucher number of specimen was deposited at the herbarium of Bu-Ali Sina University of Hamadan.

The aqueous extract was prepared according to the method described by Kim and Shin, 1998 with modification. Briefly, the plant roots were ground into a powder using electric mill, dissolved in distilled water in the ratio of 20% (W/V), and mixed for 50 min at 70°C with continuous stirring. The mixture was allowed to cool at room temperature before passing through Whatman No. 1 filter. Wherever stated the animals were treated with the plant extract

of 1 g/kg body weight by gavage for 14 days.

Preparation of tamoxifen solution

Tamoxifen (Sigma Chemical Co., USA) was dissolved at a concentration of 0.2 mg/ml in sesame oil containing 1% benzyl alcohol. For steatosisinduction in rats, each rat received subcutaneously 1 mg/kg body weight/day for 7 days according to the method described by Lien et al., 1991.

Treatments

Twenty-four adult female Wistar rats weighing 200±10 g were purchased from Pasteur Institute of Iran, IRAN. They were housed in standard cages in animal house and had free access to food and water. The room temperature was maintained at a temperature of 25±2 °C and 12 h light-dark cycle. The animals were acclimatized for at least 5 days under this condition before the start of the experiments.

The rats were randomly divided into four groups (n=6) and treated as follows:

Group-1, controls treated with vehicle. Group-2, rats received tamoxifen (1 mg/kg B.W/day, subcutaneously for 7 days. Group-3) rats received tamoxifen+chicory. Tamoxifen at a concentration of 1 mg/kg B.W/day subcutaneously, for 7 days followed by aqueous extract of chicory root extract at a concentration of 1 g/kg B.W/day, gavage, for 14 days. Group-4 rats treated with aqueous chicory root extract (1 g/kg B.W/day by gavagefor 14 days.

Blood was collect from each rat; serum was separated and used for biochemical assays. Liver tissue was removed, a portion was fixed in 10% formalin and send to pathology department for sectioning and staining by H&E staining method. One gram of liver tissue was homogenized in chloroform/methanol solution (2:1 v/v) and used for determination of triglycerides.

Biochemical assays

Biochemical assay kits were purchased from Pars Azmoon company of Iran. Glucose, cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), and total protein levels were measured in the serum of rats. Liver damage enzymes namely; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were also measured.

Triglyceride level was determined in liver homogenate using a colorimetric assay following the procedure of Folch et al., 1957 Briefly.

Histological analysis of liver tissues

A small portion of liver tissue obtained from

each rat was fixed in 10% aqueous formalin solution, washed with 70% ethanol, dehydrated using alcohol series from 70% to 100% alcohol and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin (H&E) dyes and observed under a light microscope. Histologic scoring system for non-alcoholic fatty liver disease was determined according to the method described by Kleiner et al., 2005: Score 0=5%, score 1=5-33%, score 2=33-66% and score 3=66% of affected area.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) and analyzed with SPSS software Version 16 (SPSS, Chicago, IL, USA). Comparison between groups was conducted with one way analysis of variance (ANOVA) followed by leasts significant difference (LSD) post hoc test. Results were considered statistical significant at the probability (*P*) value of 0.05.

Results

The results of this study showed that serum glucose was unaffected in the treatment groups compared to controls. However, serum total protein was decreased significantly ($P< 0.05$) in tamoxifen-treated rats compared with control group. Chicory administration by gavage tamoxifen+chicory group non-significantly increased protein concentrations (Table 1).

Although there was no significant change in the serum activities of AST and ALP between the different groups, but ALT activity elevated significantly ($P< 0.05$) in tamoxifen group compared with control group and it decreased significantly ($P< 0.05$) in tamoxifen+chicory group compared with tamoxifen group (Table 2).

Table 1 Comparison of glucose and total protein concentrations in the serum of rats in different groups.

Groups	Glucose (mg/dl)	Total Protein (g/dl)
Control	175.6 ± 17.5	8.1 ± 0.7
Tamoxifen	161.6 ± 13.8	7 ± 0.2 *****
Tamoxifen+Chicory	170.6 ± 14.6	7.3 ± 0.5
Chicory	170 ± 23.2	7.7 ± 0.7

Values are means ± SD;
*****$P< 0.05$, significantly different from Control group.

Table 2 Comparison of hepatic enzymes activity in the serum of rats in different groups.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	56 ± 5.8	91.5 ± 8.7	323 ± 52.6
Tamoxifen	77 ± 15.6 *****	118.6 ± 5	386 ± 106.1
Tamoxifen+Chicory	56.1 ± 12.4 †	140 ± 33.1	387.8 ± 199
Chicory	53.3 ± 9.5	144.3 ± 12.1	485.8 ± 275.9

Values are means ± SD;
*****$P< 0.05$, significantly different from Control group;
†$P< 0.05$, significantly different from Tamoxifen group.

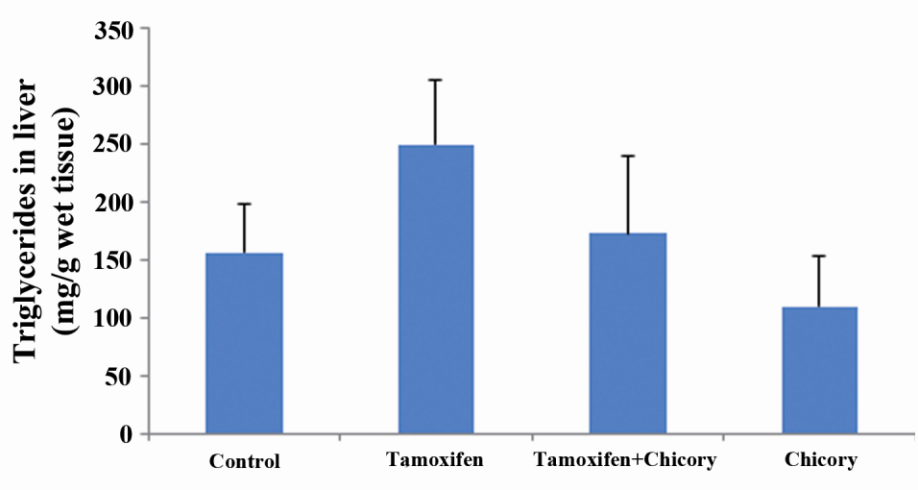

Figure 1 Effects of tamoxifen and chicory extracts on liver triglyceride levels in rats. Values are means ± SD; ***P*< 0.05, significantly different from Control group.

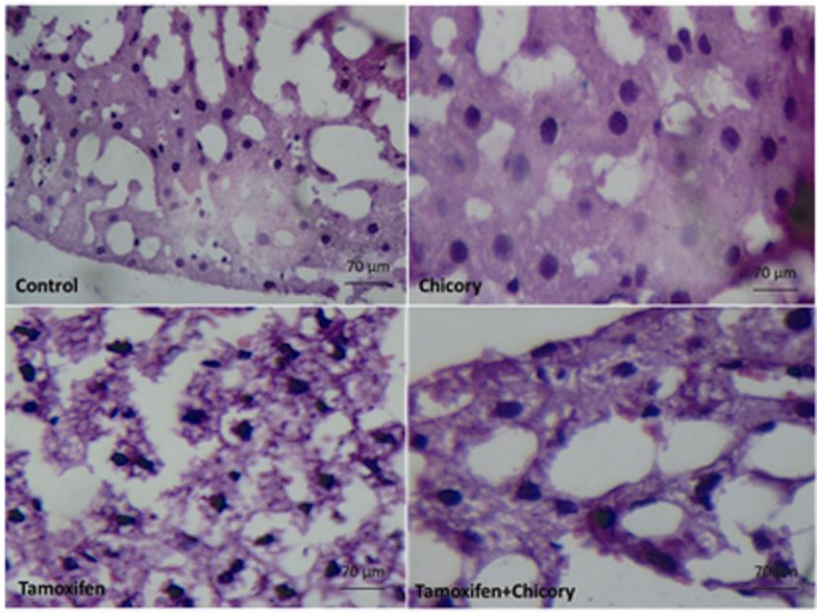

Figure 2 Histopathology of rat liver tissue in different groups. The score of steatosis were, 1 in control group, 3 in tamoxifen group, 2 in tamoxifen+chicory group and 1 in chicory group.

Comparison of the lipid profile did not show significant alteration in triglyceride and LDL-C values in different group but cholesterol and HDL-C concentrations decreased significantly (*P*< 0.05) in tamoxifen group compared with control group (Table-1)

Data presented in Figure 1, show that the concentration of triglyceride in the liver tissue of tamoxifen group was elevated significantly (*P*< 0.05) compared to control group. The

triglyceride level was partly recovered in rats treated with the plant extract (tamoxifen+ chicory group) when compared to that in tamoxifen group.

Based on histological observations rat the steatosis score wasin control group, tamoxifen group, tamoxifen+chicory group and chicory group was determined as; 1, 3, 2 and 1, respectively. The results showed that chicory extract could reverse liver steatosis score which was induced by tamoxifen (Figure 2).

Discussion

Tamoxifen is used effectively in the treatment of breast cancer (Mandlekar et al., 2000). One of the most predominant side-effects of this drug, is liver dysfunction especially fatty liver (Nishino et al., 2003). Previous studies demonstrated that breast cancer patients taking tamoxifen suffer from breast cancer also suffer from liver steatosis (Ogawa et al., 1998).

In the current research using an animal model of steatosis, we showed that tamoxifen treatments can cause liver dysfunction. A significant decrease in serum total protein along with elevation of serum ALT was the first signs of liver damage in tamoxifen-treated animals. Accumulation of triglyceride in hepatocytes that was demonstrated by biochemical and histological observations further attest to this finding. This finding was in accordance with other reports showing that tamoxifen- related changes in lipid profile (Morales et al., 1996).

Tamoxifen treatment was associated with a significant decrease in cholesterol and HDL-C compared to control group. The effect of tamoxifen on total cholesterol and LDL-C was in accordance with the report by Novoa et al. (2002) who showed that tamoxifen decreased total cholesterol and LDL-C in male patients having pubertal gynecomastia, whereas, tamoxifen treatment had no effect on triglyceride and HDL-C levels. It seems that changes in lipid profile in response to tamoxifen treatments varies depending on the dose and mode of treatments.

It has been suggested that the complications raised by conventional drugs such as tamoxifen can be prevented by consuming the medicinal plant preparations (Madani et al., 2008; Ozturk et al., 2012).In this connection, previously it has been reported that green tea and the dimethoxy biphenyl dicarboxylate possess protective and curative effects on tamoxifen-induced liver injury (El-Beshbishy, 2005). Likewise, the protective effects of milk thistle extract on fatty liver induced by tamoxifen have been reported (Behrouj et al., 2015).

Chicory is well known for its hepatoprotective, anti-diabetic and lipid lowering properties (Pushparaj et al., 2007; Madani et al., 2008; Ghamarian et al., 2012). According to Kim et al. (1998) chicory extract can improve lipid

metabolism. It has also been shown that chicory extract can ameliorate diabetes-induced fatty liver in rat models (Ziamajidi et al., 2013).The present study provides evidences for hepatoprotective action of chicory. Based on this experimental study, oral administration of chicory to tamoxifen-treated rats was effective in modulation of serum ALT and liver steatosis induced by tamoxifen.

The modulatory effects of chicory on liver steatosis is probably due to increased β-oxidation or decreased biosynthesis of fatty acids and triglycerides (Gudbrandsen et al., 2006; Cole et al., 2010; Moya et al., 2010). Also in current study chicory extract did not have any effect on the serum lipid profile. This data is justified by knowing that fatty liver did not parallel with changes in serum total triglyceride level (Cole et al., 2010).

In the other words, fatty liver patients may be did not have any hyperlipidemia. However it seems that the duration of treatment time are determining factors in these experimental setups.

In conclusion, based on this experimental study it was shown that oral administration of chicory root extract in tamoxifen-treated rats modulated tamoxifen-related hepatic damages as shown by changes in serum ALT and steatosis in the liver. It seems that the aqueous extract of this plant has beneficial effects on liver function in drug-induced liver injury, although more investigations are needed to further confirm the related pathways and mechanisms.

Acknowledgement

The author would like to acknowledge the supports provided by Hamadan University of Medical Sciences, Hamadan, Iran.

Conflict of interest

The authors declare that there are no conflicts of interest.

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