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Hepatoprotective Activity of *Silybum marianum* and *Cichorium intybus* Against Thioacetamide in Rat

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Abstract: The plant phenolic compounds such as flavonoids have an important role in the treatment of many diseases and some of them have a potent hepatoprotective effect. In this study, we have investigated the protective effects of polyphenolic extracts of *Silybum marianum* and *Cichorium intybus* on thioacethamide-induced hepatotoxicity in rat. The extracts were injected to the rats, at a dosis of 25 mg kg^{-1} body weight together whit thioacetamide at a dosis of 50 mg kg^{-1} body weight. To assess the affectivity of extracts, against thioacetamide, the activity of aminotransferases (SGOT and SGPT), alkalin phosphatase, bilirubin, Na^+ and K^+ were measured. Significant decrease in the activity of aminotransferases, alkalin phosphatase and bilirubin was observed in the groups treated with extracts and thioacetamide compared with the group that was treated only with thioacetamide. The level of Na^+, K^+ and liver weight between different groups was not significantly altered. This results prove the protective effect of extracts on liver cells. The protective effects of this extracts can be due to the presence of flavonoids compounds and their antioxidant effects.

Key words: Antioxidants, hepatoprotective, polyphenolic compounds, thioacetamide, *Silybum marianum*, *Cichorium intybus*

Introduction

The liver disorders are a world problem. Despite its frequent occurrence, high morbidity and high mortality, its medical management is currently in adequate, no therapy has successfully prevented the progression of hepatic diseases, even though newly developed drugs have been used to treat chronic liver disorders these drugs have often side effects. Therefore, that is an essential research about suitable herbal drugs, that could replace the chemical ones (Bruck *et al*., 1996).

Plant extracts have been used by traditional medical practitioners for the treatment of liver disorders for centuries (Schuppan *et al*., 1999). Phenylpropanoids or polyphenolic compounds are a large group of herbal chemical compounds with well-known treat mental and protective effects (Pyo *et al*., 2004; Marja *et al*., 1999).

Silybum marianum extracts were used as early as the 4th century B.C. for liver problems (Pyo *et al*., 2004). Its pharmacological profile is well defined and studies in cell culture and animal models clearly show its hepatoprotective property against carbon tetrachloride, paracetamol and Amanita phalloide toxin (Muriel and Mourelle, 1990; Muriel *et al*., 1992; Vogel *et al*., 1984). *Silybum marianum* seed extract has been called silymarin and consist of silybin, silychristin, silydianin and isosilybin. The flavonoid silybin constitutes 60% to 70% of silymarin (Wagner *et al*., 1974; Quaglia *et al*., 1999). Currently standardized extract from the *Silybum marianum* are used in many countries as an effective treatment for liver diseases (Pyo *et al*., 2004).

Cichorium intybus is widely used as a treatment for liver disorders. That is a potent antihepatotoxic plant and is a major component of herbal drugs (Zafar and Mujahid Ali, 1998). In some study aqueous and alcoholic extracts of this plant have shown hepatoprotective activity against carbon tetrachloride (Gazzani *et al*., 1999; Papetti *et al*., 2002).

In the present study the hepatoprotective activities of *Silybum marianum* (as positive control) and *Cichorium intybus* extracts against thioacetamid toxicity was evaluated.

Materials and Methods

Herbal extracts: *Silybum marianum* seeds and *Chicorium intybus* were collected from Isfahan Jahad Sazandegi Research farms and were identified by the Department of biology of the Isfahan university. To prepare these extracts, 100g dried herbs were initially pulverized and deaped in ethanol (once in 96%,than in 70%). The extracts were filtered and purred then decanted using chloroform and finally dried at 50°C in an oven. This dried extract was dissolved in 10 mL normal saline and prepared for injection.

Laboratory animals: Adult wistar male rat (200-250g) were obtained from the Tehran Pasteur Institute. The animals were kept at 25°C with enough humidity and under controlled lighting (12-hours light/12-hours dark) periods. The rats were fed standard diets and allowed food and water *ad libitum*.

Animals treatment: Rats were randomly divided into four groups of five animal. Each group was kept in a separate cage. Group I served as normal control and in each injection received only normal saline. Thioacetamide (Merck company, Germany) was injected intraperitonally (50 mg kg^{-1}) in 3 consecutive days for the second group (intoxicated control). In the 3rd and 4th groups, 25 mg kg^{-1} of extract, of *Silybum marianum* and *Cichorium intybus* were administered respectively with thioacetamide at dose of 50 mg kg^{-1} intraperiotenally (Amad *et al*., 2002).

Biochemical evaluation: Forty-eight hours after the last injection, the rats were anesthetized with chloroform and blood sampling was performed by cardic puncture. The collected blood samples were allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 4000 rpm for 20 min. Serum aminotransferases activities including SGOT and SGPT and also alkaline phosphatase (ALP), total bilirubin values were checked using the commercial "Man company" kits. Serum sodium and potassium values were checked using the flame photometer.

Histological examination: After blood sampling, the rats were dissected and their livers were separated. The livers were fixed with 10% formalin solution. Histolologic sections were prepared from the livers, stained and examined under light microscope.

Statistical analysis: The data were analyzed and compared by using the statistical test including analysis of variances and tukey test. P value less than 0.05 were considered as significant.

Results

Administration of thioacetamide let to significant increase of the activity of SGOT, SGPT and ALP, compared to the control group (Fig. 1, 2, 3). The level of total bilirubin was also increased (Fig. 4) but there was no significant difference in the values of sodium, potassium and liver weight percent between the groups (Fig. 5, 6).

Treatment with the polyphenolic extracts of *Silybum marianum* and *Cichorium intybus*, reduced the level of enzymes activity (SGPT, SGOT and ALP) and the level of total bilirubin, comparing with thioacetamide group. Values of sodium, potassium and liver weight shows no significant difference between these groups.

In the thioacetamide treated group, microscopic examination of the hepatic section showed that central veins of the hepatic lobules were dilated and filled with blood. Liver cells around central veins showed relatively a high number of necrosis and apoptosis. Some acute and chronic inflammatory cells also seen around the necrotic cells (Fig. 7).

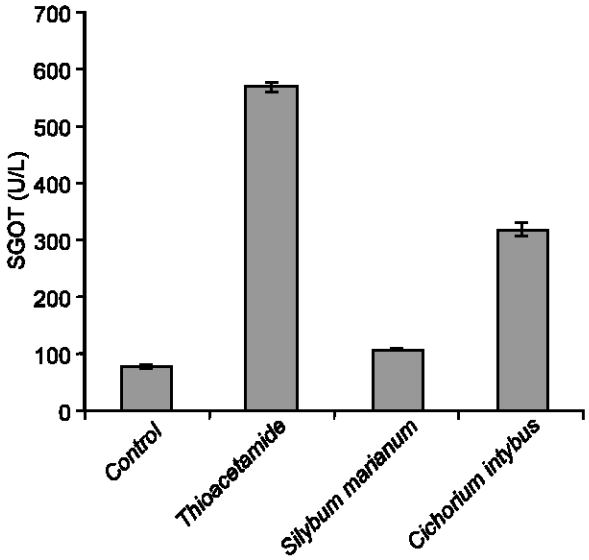

Fig. 1: The activity of SGOT

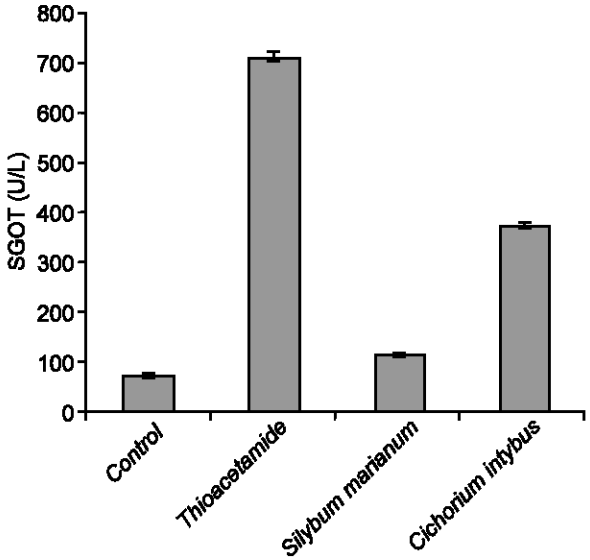

Fig. 2: The activity of SGPT

In the groups treated with polyphenolic extracts of *Silybum marianum* and *Cichorium intybus*, central veins were congested and dilted. Som apoptotic cell were also seen around the central veins. The number of these cells were much less in treated group by the *Silybum marianum*. In the remaining parts of the lobules, relatively low number of apoptosis were observed. Necrotic cells were observed very rarely in the *Silybum marianum* group (Fig. 8, 9).

Discussion

One of the major function of the liver is detoxification of xenobiotics and toxin (Mitra *et al*., 1998). In many cases,

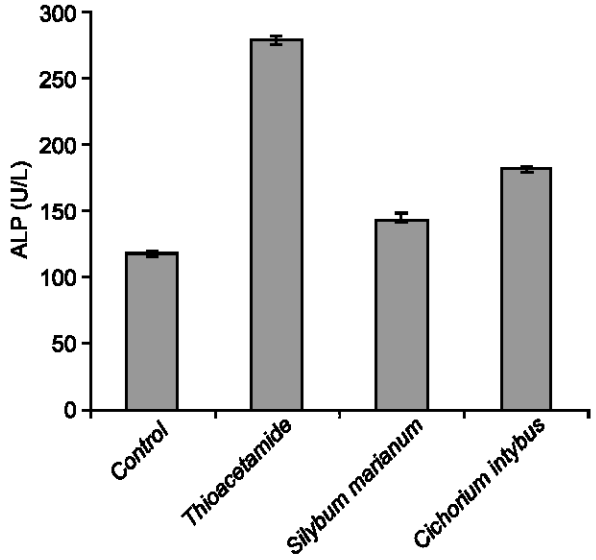

Fig. 3: The activity of ALP

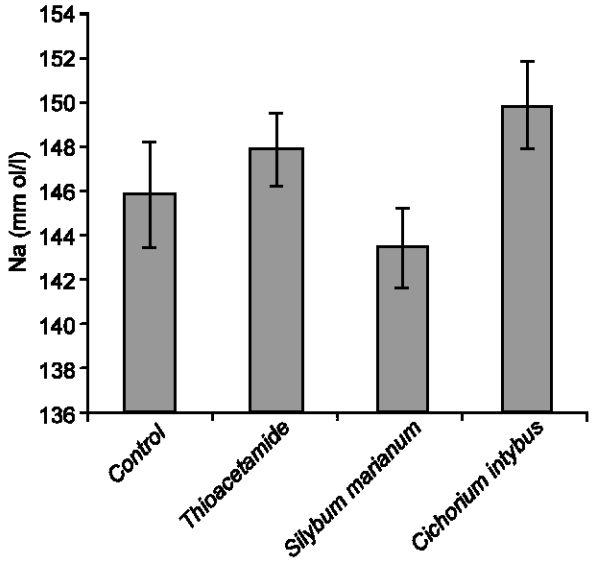

Fig. 5: The level of Na^+

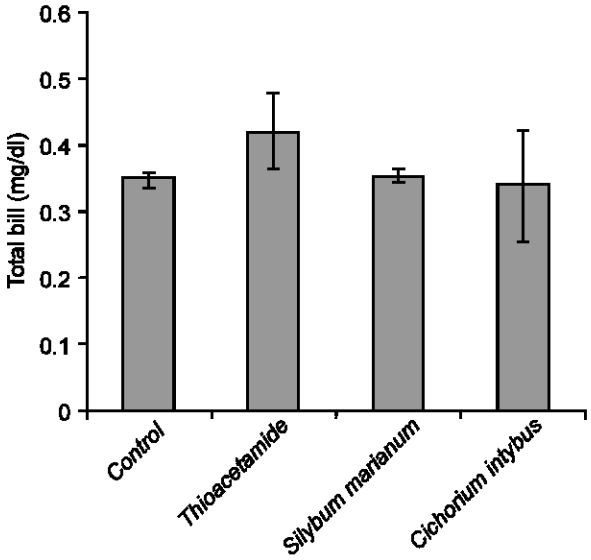

Fig. 4: The level of bilirubin

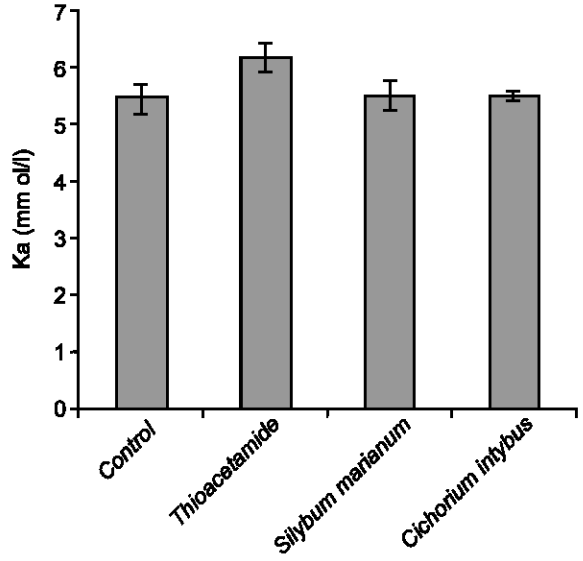

Fig. 6: The level of Ka^+

reactive oxygen species produce during detoxification (Jeong *et al.*, 1999). Over dose of toxin and some drugs such as acetaminophen or long time use of some drugs could produced large amounts of free radicals that causes oxidative stress and liver injury (Jeong *et al.*, 2002). Because liver performs many vital functions in the human body, damage of liver causes unbearable problems (Mitra *et al.*, 1998; Chattopadhyay, 2003). Thus study about hepatoprotective compounds is of importance.

Thioacetamide is a potent hepatotoxic that is metabolized by Cytp450 enzymes present in the liver microsomes and is converted to a toxic reactive intermediate called thioacetamide S-oxide due to oxidation process (Kim *et al.*, 2000; Sanz *et al.*, 1998). Thioacetamide S-oxide induced oxidative stress in the hepatic cells (Zaragoza *et al.*, 2000; Sun, 2000). It is responsible for the changes in cell permeability, increase intracellular concentration of Ca^{++}, increase in nuclear volume and enlargement of nucleoli and also inhibits mithochondrial activity wich leads to cell death and severly affecting those cells wich are located in the perivenous acinar region (Amad *et al.*, 2002; Diez-Fernandez *et al.*, 1996).

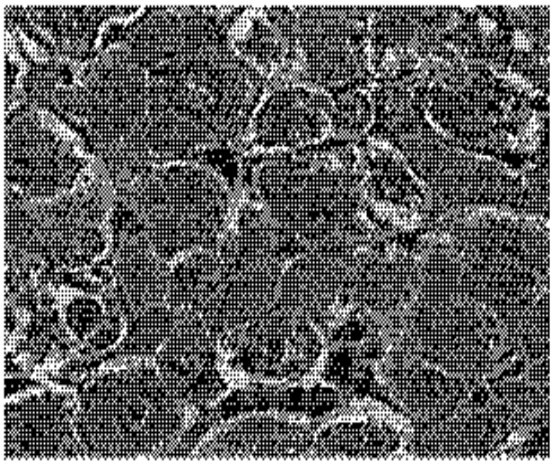

Fig. 7: Histology of the liver in the thioacetamide treated group (Magnificatin: *40)

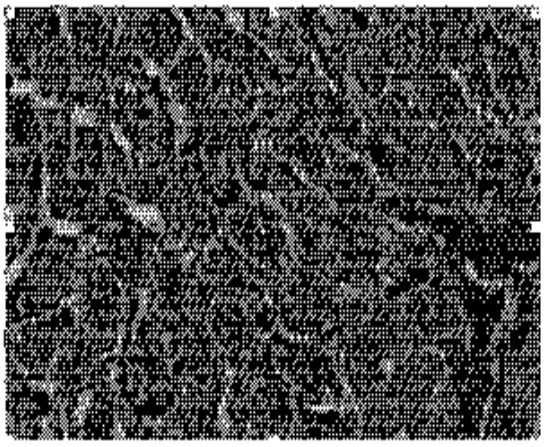

Fig. 8: Histology of the liver in the thioacetamide supplemented with *Silybum marianum* treated group (Magnificatin: *40)

Damage of liver cell is reflected by an increase in the levels of hepato specific enzymes, these are cytoplasmic in lection and are released in to circulation after cellular damage (Sallie *et al.*, 1991). In this study significant increase in the total bilirubin content and in the SGOT, SGPT and ALP activities in the thioacetamide treated group could be taken as an index of liver damage (Zafar and Mujahid Ali, 1998). Histopathological examination also confirms these result. Thioacetamide induced apoptosis and necrosis in liver cells by producing free radicals during thioacetamide metabolism (Sun, 2000). Treatment with polyphenolic extracts of *Silybum marianum* and chicorium intybus leads to a thioacetamide decrease, total bilirubin and SGOT, SGPT and ALP activities as compared with thioacetamide treated group. These confirm that polyphenolic extracts have protective effects against hepatic cell injury induced by thioacetamide.

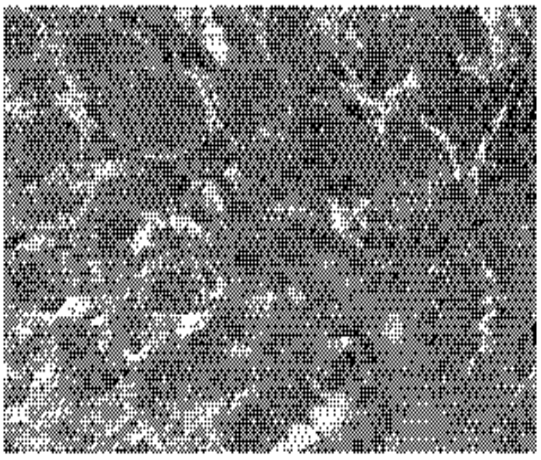

Fig. 9: Histology of the liver in the thioacetamide supplemented with *Cichorium intybus* treated group (Magnificatin: *40)

Herbal polyphenolic compounds in the cell can function as antioxidant and antiprooxidant by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions (Pyo *et al.*, 2004; Marja *et al.*, 1999; Sakihama *et al.*, 2002). This compounds and particularly silimarin, have multiple mechanisms of action, that may be hepatoprotective, including: 1) Antioxidant activity: Flavonoid in general are good free-radical scavengers. The best known antioxidants are vitamin A, vitamin C, vitamin E and the mineral selenium. However, in the liver silymarin is more that 10 times as potent as vitamin E. 2) Anti lipid prooxidation 3) Induce detoxification systems 4) protection of cell against employed glutation 5) reduction of lukoterin formation from unsaturated free acid 6) Enhanced protein synthesis 7) Stabilization of mastcell 8) Regulation of immuno functions. Also silymarin inhibit cytP450 detoxification system and prevent metabolism of toxic compound such as thioacetamide, tetrachloride and acetaminophen (Papetti *et al.*, 2002; Amad *et al.*, 2002; Mitra *et al.*, 1998; Janbaz *et al.*, 2002; Baer-Dubowska *et al.*, 1998; Zix and Agarwal, 1997).

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