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 thioacetamide-induced hepatotoxicity in rat. The extracts were injected to the rats, at a dose of 25 mg kg⁻¹ body weight together with thioacetamide at a dose of 50 mg kg⁻¹ body weight. To assess the affectivity of extracts, against thioacetamide, the activity of aminotransferases (SGOT and SGPT), alkaline phosphatase, bilirubin, Na⁺ and K⁺ were measured. Significant decrease in the activity of aminotransferases, alkaline 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 inadequate, 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 (Schupp 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, 1988). In some study aqueous and alcoholic extracts of this plant have shown hepatoprotective activity against carbon tetrachloride (Gazzani et al., 1999; Papieti et al., 2002).

In the present study the hepatoprotective activities of *Silybum marianum* (as positive control) and *Cichorium intybus* extracts against thioacetamide 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 20%, then in 70%). The extracts were filtered and purered 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.
Animal treatment: Rats were randomly divided into four groups of five animals. 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 intraperitoneally (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\(^{-1}\) intraperitoneally (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 cardiac puncture. The collected blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 4000 rpm for 20 min. Serum aminotransf erases 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. Histologic 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 sections 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](image1.png)

![Fig. 2: The activity of SGPT](image2.png)

In the groups treated with polyphenolic extracts of *Silybum marianum* and *Cichorium intybus*, central veins were congested and dilated. Some 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,
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 Cyp450 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^{2+}, increase in nuclear volume and enlargement of nucleoli and also inhibits mitochondrial activity which leads to cell death and severely affecting those cells which are located in the perivenous acinar region (Amad et al., 2002; Diez-Fernandez et al., 1996).
Herbal polyphenolic compounds in the cell can function as antioxidant and antiperoxidant by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions (Pyo et al., 2004; Menja et al., 1999; Sakihana et al., 2002). This compounds and particularly silymarin, 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) Antiperoxidation 3) Induce detoxification systems 4) Protection of cell against employed glutation 5) Reduction of lukothesis formation from unsaturated free acid 6) Enhanced protein synthesis 7) Stabilization of mastcell 8) Regulation of immune functions. Also silymarin inhibit cytochrome P450 detoxification system and prevent metabolism of toxic compound such as thioacetamide, tetrachlore and acetaminophen (Papini et al., 2002; Amad et al., 2002; Mitre et al., 1998; Janbaz et al., 2002; Baer-Dubowska et al., 1998; Zix and Agarwal, 1997).

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