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## **Hepatoprotective and Antioxidant Effects of *Silybum marianum* Plant in Rats**

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### **Abstract**

The plant phenolic compounds such as flavonoids and isoflavonoids have an important role in the treatment of many diseases and some of them induce a potent hepatoprotective effect. In the present study, the hepatoprotective and antioxidant effects of ethanolic extract of *Silybum marianum* (Milk thistle) plant were evaluated by measuring liver function; tissue antioxidant enzymes and histological examination of liver. Antioxidant enzymes as SOD, CAT and GSH were measured in liver homogenate. *In vitro* determination of the extract activity was compared to DPPH and measured spectrophotometrically. Oral administration of *Silybum marianum* extract significantly ( $P < 0.05$ ) decreased liver enzyme activity when given in repeated doses. The small and large doses increased the activity of antioxidant enzyme. The obtained results proved the protective effect of ethanolic extract on liver cells. The protective effect of this extract may be attributed to presence of flavonoids compounds and their antioxidant effects. It could be concluded that ethanol extract of *Silybium marianum* have a significant hepatoprotective and antioxidant activity and may be useful for patients who suffer from liver diseases.

**Keywords:** *Silybum marianum*; Hepatoprotective; Antioxidant; Histopathology; Rats

### **Introduction**

The liver disorders are a worldwide problem. Despite its frequent occurrence, high mortality and high morbidity, its medical management is inadequate, there are no therapy can successfully control in the progression of the disease, even though newly developed drugs has been used to treat chronic liver disorders which have often side effects. Therefore, essential researches about suitable herbal drugs that could replace the chemical one were needed<sup>1</sup>. Medicinal plants are promising source of hepatoprotective and antioxidant activity, either alone or in combination with other drugs, are valuable in the treatment of a range of liver and gallbladder disorders, poisoning from chemical and environmental toxins<sup>2</sup>. *Silybum marianum* is common plants grown in all over the globe are interesting for investigation. *it* is a member of the aster or daisy (family *Asteraceae*), which has black shiny seeds, crowned with feathery tufts like those of dandelion seeds and that seeds have been traditionally and roasted for use as a coffee substitute also used in pig, cattle, and horses as animal feeds. Their extracts were used from centuries for the treatment of liver diseases (hepatitis, cirrhosis, and icterus), against the intoxication with *Amanita* species, and also in, the case of alcoholic problems. The

hepatoprotective effect of these extracts (against hepatotoxicity of carbon tetrachloride, paracetamol, or D-galactosamine) is due to the antioxidant properties of flavonoids, to the inhibition of the synthesis of phosphatidylcholine, and stimulating of hepatic synthesis of RNA proteins. Silybin also stimulates the RNA polymerase I, and further the ribosomal RNA and synthesis of protein. Silymarin and *Silybum* species have also antiinflammatory and anticancer activities<sup>3</sup>.

**Materials and Methods**

**1.1. Plant material**

The selected plants were collected and taxonomic identifications were established by the staff members of the Department of Flora, Ministry of Agriculture. A voucher sample was kept in the Department of Pharmacology, Faculty of Veterinary Medicine, and Cairo University, Egypt. The air-dried plant material (250 g) was pulverized, and stored for further use.

**1.2. Preparation of the ethanolic extract**

Two hundred grams of the dried parts of the plant was extracted with ethanol 95% for at least 24 h, followed by percolation for 5 to 7 times till complete exhaustion. The ethanolic extract were concentrated under reduced pressure at temperature not more than 50 °C, kept at -4°C until used and the yield percentage was recorded in table 1. The extract was freshly suspended in sterile distilled water with few drops of Tween 80 to a final concentration of 100 mg/ml.

**Table 1: Plant used for screening of the hepatoprotective and antioxidant effects and the yield of ethyl alcohol extraction**

<b>Plant species</b>	<b>Plant family</b>	<b>Parts used</b>	<b>Yield / 200 g dry plant</b>
<i>Silybum marianum L</i>	<i>Asteraceae</i>	Aerial parts	48.96

**1.3. Animals**

A total of 25 mature Sprague Dawley albino rats of both sexes, weighting from 140 to 170 g were used. Rats were allocated randomly into 5 equal groups. These groups were used to test the hepatoprotective and antioxidant effect *in vivo* of oral dose (200 and 400 mg/kg b.wt.) of the tested plant. Each group was placed into a separate cage. The animals were randomly divided into 5 groups each of 5 animals. Group I (control healthy) received distilled water orally (1 ml per day) for two months. The other four groups were given CCL4 (1 ml/kg b.wt. s.c) during the last five days of the experiment. One of these groups was used as a control positive (intoxicated non treated). Group III and IV were given the ethanolic extract of *Silybum marianum* orally at doses of 200 and 400 mg/kg b.wt. daily, for two months. Group V was used as a standard group and received silymarin orally at a dose of 100 mg/kg b.wt. for two months. Blood samples were collected from the veins of orbital plexus of each animal at the end of the experimental period. Serum samples were separated by centrifugation at 3000 rpm for 10 min. These samples were used for estimating the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and histopathological examination of liver. The activity of antioxidant enzymes glutathione peroxidase, catalase and superoxide dismutase were determined in liver homogenate.

**1.4. Evaluation of the *in vitro* antioxidant activity**

The scavenging activity of (1, 1-Diphenyl, 2-picryl hydrazyl) DPPH radical was investigated according to the method described<sup>4</sup>. A methanol solution of DPPH (2.95 ml) was added to 50 µl extract sample (the tested extracts were dissolved in methanol at different concentration, 10.000 - 25 µg/ml for the ethanol extract of *Silybum marianum*) in a disposable cuvette. Ascorbic acid was used as a standard at 0.1 M concentration which equal to 17613 µg/ml as described<sup>5</sup>. The absorbance of the standard and samples were measured at 517 nm at regular interval of 15 sec for 5 min. The inhibition percent for each sample was calculated using the following formula:

$$\begin{matrix} \% \text{ inhibition} \\ \text{(reactive reaction rate)} \end{matrix} = \frac{\text{Abs. (DPPH solution)} - \text{Abs. ( Sample)}}{\text{Abs.(DPPH solution)}} \times 100$$

**1.5. Serum analysis**

The enzymatic determination of catalase activity according to the method<sup>6</sup>, superoxide dismutase activity according to method described<sup>7</sup>, glutathione peroxidase activity according to method described<sup>8</sup>. Alkaline phosphates activity (ALP) according to the method<sup>9</sup>, AST or ALT activity in serum according to the method<sup>10</sup> and Histopathological examination according to the method described<sup>11</sup>.

**1.6. Statistical analysis**

The data were expressed as mean± Standard deviation (S.D.).Differences between means in different groups were tested for significance using a one-way analysis of Variance (ANOVA) followed by Duncan’s multiple range test. Differences were considered significant at level  $P < 0.05$  according to<sup>12</sup> using SPSS program version 15.

**RESULTS**

**2.1. Determination of hepatic antioxidant enzymes (CAT, SOD, GSH- px):**

The antioxidant activity of ethanol extract of *Silybum marianum* was studied in liver homogenate of rats after prolonged oral administration (2 months) via determination of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) enzyme activity. The ethanol extract significantly stimulated the antioxidant activity in liver homogenate of the treated rats as evident by the increased levels of the tested antioxidant enzymes (Table 2).

**Table (2):** Effect of the administration of ethanol extract of *Silybum marianum* for 2 months on the levels of (SOD), (GSH-px), and (CAT) in liver homogenate of rats (n=5).

Group	Dose (mg/kg b.wt.)	SOD (U/g)	GSH-px (U/g)	CAT (U/g)
Control	0	188.00±0.577 <sub>a</sub>	0.117±0.000 <sup>a</sup>	0.14±0.006 <sub>a</sub>
Ethanol extract of <i>Silybum marianum</i>	200	285.47±2.886 <sub>b</sub>	0.175±0.003 <sup>b</sup>	1.29±0.095 <sub>c</sub>
	400	321.00±3.464 <sub>d</sub>	0.233±0.006 <sup>d</sup>	1.75±0.148 <sub>d</sub>

## ANTIOXIDANT EFFECTS OF *SILYBUM MARIANUM*

The effect of ethanol extract of *Silybum marianum* at doses of 200 and 400 mg/kg b.wt on liver enzymes (ALT, AST, and ALP) was reported in Table (3). CCL4 elevated the ALT level in the intoxicated group as compared to of the control (non treated) group. Rats pretreated with ethanol extract of *Silybum marianum* at doses 200 and 400 mg/kg b.wt. for 2 months significantly protected the liver and decreased the ALT as compared to CCL4 intoxicated group. Silymarin significantly decreased the enzyme activity. The CCL4 intoxicated group elevated the AST level compared to the control (non treated) group. The ethanol extract of *Silybum marianum* at doses of 200 and 400 mg/kg b.wt. for 2 months significantly decreased AST level. Silymarin significantly decreased AST enzyme level. The level of ALP enzyme was elevated in the intoxicated rats compared to the control (non treated) group. The ethanol extract of *Silybum marianum* at doses 200 and 400 mg/kg b.wt. for 2 months significantly decreased ALP enzyme levels compared to control (non treated) group. Silymarin was significantly decreased ALP enzyme level.

**Table (3): Effect of the ethanol extract of *Silybum marianum*, and Silymarin (standard) for 2 months on the serum activity of ALT, AST, and ALP in CCL4-intoxicated rats (n=5).**

Groups	Dose mg/kg b.wt.	ALT (U/ml)	AST (U/ml)	ALP(U/ml)
Control (non treated)	0	82.8±2.24 <sup>b</sup>	133.4±1.96 <sup>a</sup>	135.8±2.63 <sup>a</sup>
CCL4 control (intoxicated)	0	124.4±5.00 <sup>c</sup>	263.0±7.68 <sup>c</sup>	229.4±1.91 <sup>c</sup>
Ethanol extract of <i>Silybum marianum</i>	200	76.8±4.16 <sup>ab</sup>	148.0±4.96 <sup>b</sup>	147.6±2.58 <sup>b</sup>
	400	72.4±4.62 <sup>ab</sup>	134.2±2.59 <sup>a</sup>	136.6±2.87 <sup>a</sup>
Silymarin (standard)	100	68.8±3.26 <sup>a</sup>	136.6±1.89 <sup>ab</sup>	135.4±1.86 <sup>a</sup>

### 2.2. Histopathological examination:

Microscopically, the liver of control non treated rats revealed normal architecture of hepatic lobules. The central veins, portal tract, hepatocytes, and sinusoids appear normal. The lobular unit was well identified as shown in Figure (1). Liver of CCL4- intoxicated rats showed loss of the normal liver architecture. There were a vacuolar degeneration of hepatocytes and individual hepatocellular necrosis (Figure 2). Focal oval cell hyperplasia between hepatic cords associated with individual hepatocellular necrosis and binucleated hepatocytes were demonstrated in Figure 3. Histopathological examination of silymarin treated rats revealed normal size and shape of hepatocytes with large rounded vesicular nuclei and increase number of binucleated cells as shown in Figure 4. Microscopical examination of liver sections from rats pretreated with the ethanol extract of *Silybum marianum* at dose 200 mg/kg b.wt. for 2 month decrease the severity of histopathological changes induced by CCL4 (intoxicated) group. Liver showed normal hepatocytes and sinusoids except in some examined sections showed minute focal area of hepatocellular necrosis replaced with mononuclear cells with vascular degeneration of the surrounding hepatocytes (Figure 5). The liver of rats given large dose (400 mg/kg b.wt.) of *Silybum marianum* extract showed normal hepatocytes, hepatic cord, and sinusoids as well as kupffer cell activation. Hepatocellular necrosis was focal and very minute with infiltrations with mononuclear cells only detected in only one section (Figure 6).

**2.3. In vitro evaluation of antioxidant activity using DPPH**

Antioxidant activity of *Silybum marianum* ethanol extract was evaluated *in vitro* as free radical scavenger activity.

The reactive reaction rates (inhibition %) with a mean value ± standard error of *Silybum marianum* were 96.14±0.16, 56.77±2.09 and 17.80±0.54 % at concentrations of 10000, 1000, and 25 µg/ml of methanol solution of the plant extract, respectively as shown in Table (3).

**Table (3):** Showing reaction reactive rate (Inhibition %) of different concentrations of ethanol extract of *Silybum marianum* at different time intervals as compared with ascorbic acid (standard).

Tested material	Concentration (µg/ml)	Reaction reactive rate (Inhibition %)
Ascorbic acid (Standard)	17613	99.12±0.16 <sup>a</sup>
Ethanol extract of <i>Silybum marianum</i>	10000	96.14±1.66 <sup>a</sup>
	5000	76.56±3.25 <sup>b</sup>
	1000	56.77±2.09 <sup>c</sup>
	100	27.67±0.28 <sup>d</sup>
	50	21.00±0.16 <sup>e</sup>
	25	17.80±0.54 <sup>e</sup>

**Discussion**

In the present study, oral administration of *Silybum marianum* extract for 2 month before CCL4 intoxication offered protection to the rat liver evidenced by the histopathological examination to the pretreated liver sample which was near to the normal hepatocytes and sinusoids. In addition, serum biochemical parameters as (AST, ALT, and ALP) were also increased compared to the control intoxicated group. This finding was consistent with those previously recorded<sup>13</sup>, which concluded that the ACTIVAlloe complex (mixture of *Aloe Vera* and *Silybum marianum*) has hepatoprotective effects in both acute and chronic liver injuries induced by CCL4. Also,<sup>14</sup> found that the ethanol extract of *S. marianum* were significantly decrease the liver enzymes after CCL4 and noticed some equal improvements in the histopathological studies for the protective groups with the extract. Finally they concluded that *Silybum marianum* got a bright reputation in relieving the liver diseases that might be due to potent silymarin mixture and its mechanism of action mainly as antiradical and anticarcinogenic roles.

The antioxidant activity of ethanol extract of the plant extract was proven by a significant increase in the levels of all the antioxidant enzymes (CAT, GSH and SOD) in liver homogenates in rats. In this concern,<sup>2,15</sup> reported that flavonoids of *Silybum marianum* had potent antioxidant effect, indicated by significant increase of superoxide anions, and lipid oxygen radicals due to lipid peroxidation as proven in this study.. Several investigators previously reported the potent *in vivo* antioxidant activity of *Silybium marianum*,<sup>14,16</sup>, they referred its *in vivo* antioxidant activity via increasing the levels of glutathione, which is an important antioxidant that detoxifies an array of hormones, drugs and chemicals. In addition,<sup>17</sup> mentioned their glutathione enhancer and liver regenerator effects as the result obtained from this study.

## ANTIOXIDANT EFFECTS OF *SILYBUM MARIANUM*

There is a growing interest in the antioxidant properties of many herbs and spices that were reported to be effective in retarding the process of lipid peroxidation in oils and fatty acids<sup>18,19,20</sup>, Concerning the antioxidant activities of *Silybum marianum*. It was found the plant extract had high free radical scavenger activity expressed as a reactive reaction % at a dose dependant manner. However, it was less potent than the ascorbic acid activity. In this concern, <sup>2,15</sup>, reported that flavonoids of *Silybum marianum* had potent antioxidant effect, capable of scavenging free radicals, superoxide anions, and lipid oxygen radicals due to lipid as proven in this study. Several investigators previously reported the potent *in vitro* antioxidant activity of *Silybum marianum* such as <sup>16, 21, 22, 23, 24</sup>.

In the present study, the elevation of GSH levels in blood and liver was observed in the *Silybum marianum* treated rats. This indicates that these plants can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H<sub>2</sub>O<sub>2</sub> and molecular oxygen<sup>25</sup>, hence diminishing the toxic effects caused by their radical.

### Conclusion

In conclusion, our results demonstrated that ethanol extract of *Silybum marianum* has a potent hepatoprotective and *in vivo* and *in vitro* antioxidant effects. The hepatoprotective effect of plant may be due to the decreased liver enzyme levels with significant improvement to the histological picture of liver. The activity of antioxidant enzymes are significantly increased in pretreated extract rat liver homogenate. Inhibition (%) of reaction reactive rate by *Silybum marianum* extract *in vitro* confirms that it is a potent free radical scavenger.

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