Hepatoprotective and Antioxidant Effects of *Silybum Marianum* Plant against Hepatotoxicity Induced by Carbon Tetrachloride in Rats

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Abstract: The acute toxicity of ethanolic extract of *Silybum marianum* (SM) plant seeds and its effect on body weight were studied in rats. *In vitro* determination of the antioxidant activity of SM extract using 1, 1- diphenyl 2-picryl hydrazyl (DPPH) radical was carried out. The hepatoprotective and *in vivo* antioxidant effects of SM extract were evaluated in CCL4 - intoxicated rats. Estimation of serum liver enzymes (ALT, AST and ALP), antioxidant enzymes (SOD, GPx and CAT) in hepatic tissue and liver histopathology were the parameters used in the study. The results showed that no mortalities occur when SM extract was orally given to rats in graded doses up to 8.0 g b.wt. kg^{-1}. SM extract induced a significant *in vitro* antioxidant activity when compared to standard ascorbic acid. Pretreatment of CCL4 – intoxicated rats with SM extract (200 and 400 mg/kg^{-1} b.wt./day) for 8 weeks significantly ($P< 0.05$) decreased the elevated serum liver enzymes and increased the activity of antioxidant enzymes in liver homogenate when compared to the CCL4-poisoned group. These biochemical findings were accompanied by amelioration of hepatic degenerative changes (vacuolar degeneration and necrosis) induced by CCL4. The results proved the protective effect of *Silybum marianum* on liver cells. The protective effect of SM extract may be attributed to the antioxidant effect flavonoids present in this plant. It could be concluded that *Silybium marianum* plant have high safety, hepatoprotective effect and antioxidant activity in rats. The study recommends that intake of *Silybium marianum* plant may be beneficial for patients who suffer from liver diseases associated with oxidative stress.
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1. Introduction

Medicinal plants are promising natural source of hepatoprotective and antioxidant compounds and are valuable in the treatment of liver and gallbladder disorders and in the protection against poisoning from chemical and environmental toxins (Muriel *et al.*, 1990). The phenolic compounds such as flavonoids and isoflavonoids which are found in many medicinal plants have been proved to play an important role in the treatment of many diseases. The liver diseases are a worldwide problem. Despite of its frequent occurrence, high mortality and high morbidity, its medical management is still inadequate. Therefore, essential researches about suitable and effective herbal drugs that could replace the chemical one are needed (Bruck *et al*., 1996).

Carbon tetrachloride (CCL4) is a selective hepatotoxic chemical agent. CCL4 produced reactive free radicals (CCl3) which initiate cell damage through two mechanisms of covalent binding to the membrane proteins and cause lipid peroxidation (Parola *et al*., 1992). Production of free reactive radicals by reductive metabolism of CCL4 is believed to cause lipid peroxidation which is associated with hepatic cell damage and leads to liver cirrhosis and fibrosis (Badary *et. al.*, 2003). These toxic effects were partially prevented by antioxidant compounds such as silymarin, α - tocopherol and salvianolic acid (Mourelle *et al.*, 1989, Parola *et al*., 1992 and Hu *et al.*, 1997).

Silybum marianum (Family *Asteracae*), is a common plant grown allover the globe which has black shiny seeds. These seeds have been roasted for use as a coffee substitute and also used in pigs, cattle, and horses as animal feeds. Extracts of *Silybum marianum* seeds were used in folk medicine for the treatment of liver diseases (Ramasamy and Agarwal, 2008). Silibinin from *Silybum marianum* was reported to produce anticancer activity against breast cancer (Kim *et al*., 2009). Silymarin from *Silybum marianum* have also anti-apoptotic, anti-inflammatory and hepatoprotective effects and therefore it may be beneficial for treatment of experimental hepatitis (Aghazadeh *et al.*, 2011).

The present study was undertaken to determine the acute oral toxicity and to evaluate hepatoprotective and antioxidant effects of the ethanolic extract of *Silybum marianum* plant seeds in CCL4 - intoxicated rats.

2. MATERIAL and METHODS

2.1. Plant:

Silybum marianum seeds were purchased from a local market of Agricultural Seeds, Spices and

Medicinal Plants, Cairo, Egypt. Taxonomic identification of these seeds was established by the staff members of the Department of Botany, Faculty of Agriculture, Cairo University. The air - dried seeds (500 g) were grinded into a fine powder and kept for further use.

2.2. Rats:

Sexually mature male Sprague Dawley rats weighing 170 -180 g body weight and 12-14 weeks old were used. Animals were obtained from the Laboratory Animal Colony, Helwan, Egypt. Rats were housed in a well ventilated animal room under standard conditions of 24 ± 3 ° C temperature, relative humidity 50 5% and 12 hr light/12 hr dark cycle at the Animal House in Agricultural Research Center, Ministry of Agriculture, Giza Egypt. Basal diet and water were provided *ad libitum* and rats were acclimatized to the laboratory environment for 7 days before start of the experiment.

2.3. Preparation of basal diet:

Basal diet was prepared according to the method of Reeves *et al.* (1993). It is consisted of 20 % protein (casein), 10 % carbohydrate (sucrose), 4.7% fat (corn oil), 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100 %.

2.4. Preparation of the extract:

The extract of *Silybum marianum* seeds was prepared according to the method described by Shalaby and Hamowieh (2010). Two hundred grams of the dried powder of *Silybum marianum* seeds were soaked in 1 liter of 70% ethyl alcohol and kept in a refrigerator with daily shaking for 5 day. This was followed by percolation for 5 to 7 times till complete exhaustion. The ethanolic extract was then concentrated under reduced pressure using a vacuum rotatory evaporator (Model 750, manufactured in West Germany) at 50 C temperature. After evaporation of ethanol, the amount of semisolid extract yielded was 48.6 grams Twenty grams of the obtained semisolid extract were suspended in 98 ml distilled water and 2 ml of Tween 80 (suspending agent) to obtain 20% liquid extract (final concentration of 200 mg/ml). The extract was kept in a refrigerator at - 4 C and till its use for the study.

2.5. Acute toxicity experiment:

A preliminary experiment was performed to determine the beginning of non lethal dose (LD_0). To determine the acute LD_{50} of ethanolic extract of *Silybum marianum* extract, forty male rats were randomly distributed into 8 equal groups, each of 5 animals. Rats were given orally the ethanolic extract of *Silybum marianum* in graded doses from 4 to 12 g kg^{-1} b.wt. Toxic symptoms and number of rats which died in each group after 48 hrs observation were recorded. The oral LD_{50} of the extract was calculated by mathematical method as described by Gad and Weil (1982).

2.6. *In vitro* antioxidant activity:

The scavenging activity of 1, 1-Diphenyl, 2-picryl hydrazyl (DPPH) radical was investigated according to the method described by Peiwu *et al.* (1999). In brief, a methanolic solution of DPPH at volume of 2.95ml was added to 50 µl of different concentrations of *Silybum marianum* extract samples (total volume equal 3ml) in a disposable cuvette. Ascorbic acid was used as a standard at 0.1 M concentration which equal to 17613 µg/ml as described by Govindarajan *et al.* (2003). The absorbance of the standard and extract samples was measured at wave length 517 nm at regular interval of one minute for 5 minutes. The inhibition percent for each sample was calculated using the following formula:

$$\text{Inhibition (\%) of reaction rate} = \frac{\text{Abs. (DPPH Sol.)} \quad \text{Abs. (Extract sample)}}{\text{Abs. (DPPH Sol.)}} \times 100 \qquad \textit{Equation (1)}$$

2.7. Effect on body weight and feed efficiency:

A total of 28 rats were randomly distributed into 4 equal groups each of 7 animals. All rats were fed on basal diet throughout the experiment period and one group was kept as normal control (non treated). The other 3 groups were orally given ethanolic extract of *Silybum marianum* extract in doses 200, 400 and 800 mg kg^{-1} b.wt. once daily for 8 weeks, respectively. Daily feed intake was calculated during the experimental period and body weight gain percent and feed efficiency were calculated according to Chapman *et al.* (1959).

2.8. Hepatoprotective and *in vivo* antioxidant activity:

A total of 35 mature male Sprague Dawley rats were allocated randomly into 5 equal groups, each of 7 rats. Group I (normal control) received distilled water orally (1 ml / day) for 8 weeks. The other four groups were injected S/C with 1 ml/kg b.wt. of CCL4 (El-Gomhoryia Company for Chemical Industries, Cairo, Egypt) during the last week of the experiment to induce acute liver damage according to Nadeem *et al.* (1996). One of these groups was kept as a positive control (intoxicated non treated). Groups III and IV

were given the ethanolic extract of *Silybum marianum* orally at doses of 200 and 400 mg/kg b.wt. daily for the same period. Group V was used as a standard and received purified silymarin (supplied by Sigma Chemical Co., St Louis, MO, USA) orally at a dose of 100 mg/kg b.wt. for 8 weeks. At the end of experiment period, blood samples were collected for serum separation by centrifugation at 3000 rpm for 10 min. Serum samples were used for estimating the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) enzymes. Halves of livers of the sacrificed rats were used for preparation of liver homogenates for estimation of the activity of antioxidant enzymes (SOD, GPx and CAT). The other halves of livers were taken for histopathological examination.

2.9. Preparation of liver homogenate:

One gram of liver tissues was collected from each rat at the end of experiment. Liver tissue was washed by ice-cold 0.9% NaCl solution and homogenized in ice-cold 1.15% solution of potassium chloride in 50 mM potassium phosphate buffer solution (pH 7.4) to yield a 10% (W/V) homogenate. Homogenization was carried out using Sonicator 4710 Ultrasonic Homogenizer (Cole-Parmer Instrument Company., USA). The homogenate was centrifuged at 4000 rpm for 5 min at 4° C. The supernatant was collected and used for determination of the concentration of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

2.10. Biochemical analysis:

The collected serum samples were used for estimation of alanine and aspartate aminotransferases (ALT and AST) according to the method of Reitman and Frankel (1957) and alkaline phosphates (ALP) activity according to the method of Kind and king (1954). In liver homogenates, the enzymatic determination of superoxide dismutase (SOD) activity was carried out as described by Nishikimi *et al.* (1972); glutathione peroxidase (GPx) activity as described by Paglia and Valentine (1967) and catalase (CAT) activity was performed according to the method described by Sinha (1972).

2.11. Histological procedure:

Halves of livers of the sacrificed rats were taken and fixed in 10 % neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were then cleared in xylene, embedded in paraffin boxes, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H&E). These sections were then microscopically examined using light microscope as described by Carleton (1976).

2.12. Statistical analysis

Data were presented as mean ± Standard deviation (SD). Differences between means in different groups were tested for significance using a one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests. Differences were considered significant at probability level $P<0.05$ according to Snedecor and Cochran (1986) using computerized SPSS program.

3. Results:

3.1. Acute toxicity:

The results showed that the calculated oral LD_{50} of ethanolic extract of *Silybum marianum* (SM) seeds was 9.25 g kg^{-1} b.wt. No toxic symptoms and mortalities were reported following administration of the extract to rats in graded doses up to 8 g kg^{-1} b.wt. during 48 hrs post-administration. This result indicates that SM extract is safe in rats.

3.2. Effect on body weight:

Oral administration of ethanolic extract of Silybum *marianum* (SM) at 3 doses (200, 400 and 800 mg kg^{-1} b.wt.) for 8 weeks did not cause significant ($P < 0.05$) changes in daily feed intake, body weight gain (%) and feed efficiency ratio (FER) when compared with the normal control rats. The body weight gain (%) and FER were 35.70 ± 0.09 % and 0.13 ± 0.007 respectively in normal control rats. In rats given SM extract, the body weight gain (%) ranged from 34.80 ± 0.08 to 35.40 ± 0.07 % and FER ranged from 0.11 ± 0.002 to 0.14± 0.009.

3.3. *In vitro* antioxidant activity:

In vitro determination of antioxidant activity using 1, 1-diphenyl 2-picryl hydrazyl (DPPH) radical showed that inhibition percents of the reaction rate (mean ± SD) of *Silybum marianum* (SM) ethanolic were 17.80±0.54, 56.77±2.09 and 96.14±0.16 % at concentrations of 25, 1000, and 10000 µg/ml of the extract, respectively. Ascorbic acid induced the highest inhibition percent of reaction rate (99.12± 0.16). This result denotes that the antioxidant activity of SM extract was dependant on its concentration. Ascorbic acid was more effective than SM extract (Table 1).

Table (1): *In vitro* inhibition percents of the reaction rate with DPPH radical by different concentrations of *Silybum marianum* extract when compared to ascorbic acid.

Tested materials	Concentration (µg /ml)	Inhibition of the reaction rate (%)
Ascorbic acid (Standard)	17613	99.12 ± 0.16 [a]
Ethanolic extract of *Silybum marianum*	25	17.80 ± 0.54 [e]
	50	21.00 ± 0.16 [e]
	100	27.67 ± 0.28 [d]
	1000	56.77 ± 2.09 [c]
	5000	76.56 ± 3.25 [b]
	10000	96.14 ± 1.66 [a]

Data denote means ± SD
Means with different letter superscripts in each column are significant at $P < 0.05$ using one way AVOVA test.
n= 5 readings for each tested sample.

3.4. Serum liver enzymes:

The effect of ethanolic extract of *Silybum marianum*(SM) seeds on the activity of serum liver enzymes (ALT, AST and ALP) is recorded in Table (2). Subcutaneous injection of CCL4 to rats in a dose 1 ml/kg b.wt. significantly ($P < 0.05$) elevated levels of ALT, AST and ALP enzymes in the serum when compared with the control (non intoxicated) group. Rats pretreated with the ethanolic extract of SM in doses 200 and 400 mg/kg b.wt. had significantly decreased ALT, AST and ALP levels in the serum when compared to CCL4- intoxicated rats. Pretreatment with silymarin (standard) in a dose 100 mg/kg b.wt. significantly ($P < 0.05$) decreased the activity of serum liver enzymes when compared to CCL4- intoxicated rats (Table 2).

Table (2): Effect of oral administration of *Silybum marianum* extract for 8 weeks on serum levels of ALT, AST and ALP enzymes in CCL4 - intoxicated rats.

Groups	Dose (mg/kg b.wt.)	ALT (U/ml)	AST (U/ml)	ALP (U/ml)
Normal control	0	82.8 ± 2.24[b]	173.4 ± 1.96[b]	155.8 ± 2.63[b]
Positive control (CCL4)	1ml	124.4 ± 5.00[a]	263.0 ± 5.68 [a]	212.4 ± 1.91[a]
Ethanolic extract of *Silybum marianum*	200	76.8 ± 4.16[c]	148.0 ± 4.96[c]	147.6 ± 2.58[c]
	400	72.4 ± 4.62[c]	144.2 ± 3.59[c]	142.6 ± 2.87[c]
Standard (Silymarin)	100	68.8 ± 3.26[d]	122.6 ± 1.89[d]	135.4 ± 1.86[d]

Data denote means ± SD
Means with different letter superscripts in each column are significant at P < 0.05 using one way AVOVA test
n=7 rats.

3.5. *In vivo* antioxidant activity:

Data showed that subcutaneous injection of CCL4 to rats (1ml /kg b.wt.) significantly ($P < 0.05$) decreased levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes in liver homogenate when compared with the control group. Pretreatment of rats with the ethanolic extract of SM (200 and 400 mg/kg b.wt.) and silymarin (100 mg/kg b.wt.) for 8 weeks significantly ($P < 0.05$) increased the levels of antioxidant enzymes when compared with CCL4- intoxicated rats (Table 3).

Table (3): Effect of oral administration of *Silybum marianum* extract for 8 weeks on the activity of SOD, GPx and CAT enzymes in liver homogenate of CCL4- intoxicated rats.

Groups	Dose (mg/kg b.wt.)	SOD (U/g)	GPx (U/g)	CAT (U/g)
Normal control	0	188.00 ± 0.577 [d]	0.117 ± 0.001 [d]	0.145 ± 0.006 [d]
Positive control (CCL4)	1ml	105.00 ± 0.266 [e]	0.012 ± 0.001 [e]	0.115 ± 0.006 [e]
Ethanolic extract of *Silybum marianum*	200	285.47 ± 2.886 [c]	0.175 ± 0.003 [c]	1.294 ± 0.095 [c]
	400	321.00 ± 3.464 [b]	0.233 ± 0.006 [b]	1.715 ± 0.048 [b]
Standard (Silymarin)	100	335.00 ± 1.245 [a]	0.333 ± 0.006 [a]	1.925 ± 0.022 [a]

Data denote means ± SD
Means with different letter superscripts in each column are significant at P < 0.05 using one way AVOVA test
n=7 rats.

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3.6. Histopathological findings:

Microscopically, examination of liver sections of normal control (non intoxicated) rats revealed normal architecture of hepatic lobule unit (central veins, portal tract, hepatocytes and sinusoids) as shown in Figure (1). Liver of CCL4 - intoxicated rats showed loss of the normal liver architecture. There was vacuolar degeneration of hepatocytes, sinusoidal congestion and individual hepatocellular necrosis (Figure 2). Focal oval cell hyperplasia between hepatic cords associated with hepatocellular necrosis were also seen (Figure 3). Histopathological examination of livers of silymarin pretreated - rats revealed normal size and shape of hepatocytes with large rounded vesicular nuclei and increase number of binucleated cells as shown in Figure (4). Microscopically, examination of liver sections of rats pretreated with the ethanolic extract of *Silybum marianum* in a daily dose 200 mg/kg b.wt. for 8 weeks decrease the severity of histopathological changes induced by CCL4. The liver showed normal hepatocytes and sinusoids except in some examined sections showed minute focal area of hepatocellular necrosis replaced with mononuclear cells (Figure 5). The liver of pretreated rats with large dose (400 mg/kg b.wt.) of *Silybum marianum* extract showed normal hepatocytes, hepatic cord, and sinusoids as well as kupffer cell activation (Figure 6).

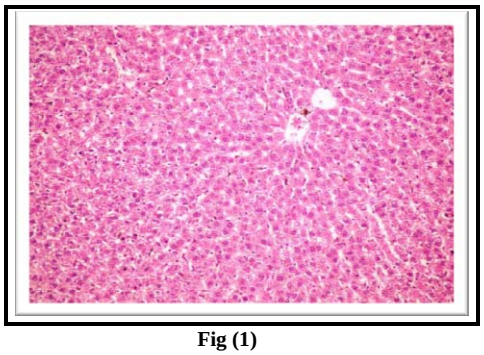

Fig (1)

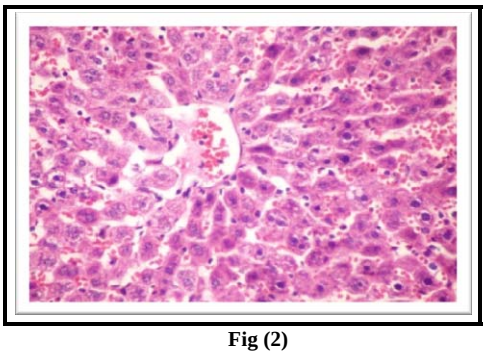

Fig (2)

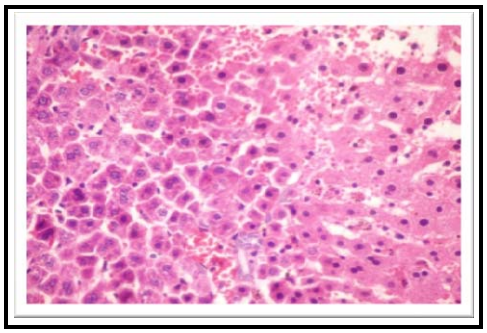

Fig (3)

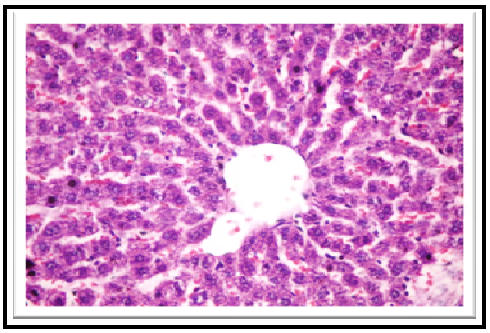

Fig (4)

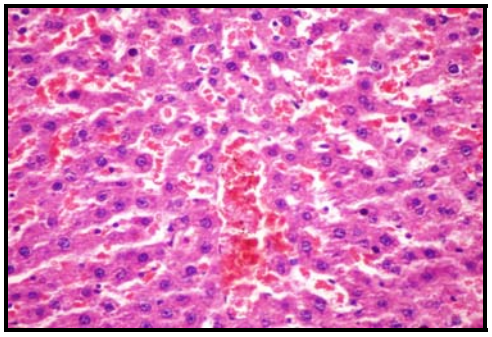

Fig (5)

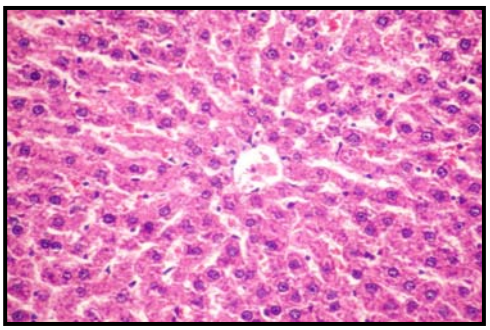

Fig (6)

Fig. (1): Liver section of a normal control rat showing normal architecture with normal central veins, portal tract, hepatocytes and sinusoids. (H and E X 400).

Fig. (2): Liver section of a rat given CCL4 showing vacuolar degeneration of hepatocytes, sinusoidal congestion and individual hepatocellular necrosis. (H and E X 400).

Fig. (3): Liver section of a rat given CCL4 showing coagulative necrosis and oval cell hyperplasia. (H and E X 400**)**

Fig. (4): Liver section of a rat pretreated orally with silymarin 100 mg/kg b.wt. for 8 weeks then exposed to CCL4 showing normal size and shape of hepatocytes with large rounded vesicular nuclei and increased number of binucleated cells. (H and E X 400)

Fig. (5): Liver of a pretreated with *Silybum marianum* at 200 mg/kg for 8 weeks showing mild congestion of hepatic central vein and sinusoids. (H and E X 400)

Fig. (6): Liver of a rat pretreated with ethanol extract of *Silybum marianum* at a dose of 400 mg /kg b.wt. for 8 weeks then exposed to CCL4 showing normal hepatocytes, hepatic cord, and sinusoids as well as kupffer cell activation. (H and E X 400).

4. Discussion

In the current study, the acute oral LD_{50} of ethanolic *extract of Silybum marianum* (SM) seeds was determined and its effect on body weight and feed efficiency ratio was examined in rats as well as its hepatoprotective and antioxidant activities were evaluated in carbon tetrachloride (CCL4) - intoxicated rats.

The results revealed that oral LD_{50} of ethanolic extract of SM was 9.25 g kg^{-1} b.wt. in male rats. This finding denotes that SM plant has a high safety in rats as no toxic symptoms and mortalities were reported when rats were given its extract up to 8 g /kg b.wt. However, Gad and Weil (1982) mentioned that when LD_{50} of a compound exceeds 5 g /kg b.wt. it is considered safe. The high safety of SM extract was similar to that reported by Post-White *et al*. (2007) who concluded that Milk thistle (*Silybum marianum*) is considered safe and well tolerated, with mild gastrointestinal upset and rare allergic reactions when used in man. In rats the same authors reported that SM plant is safe when orally given in doses up to 11.6 g/ b.wt. for males and 9.75 g/ b.wt. for females. Moreover, Tamayo and Diamond (2007) reviewed safety and efficacy of SM plant and concluded that it is safe and well tolerated, and toxic or adverse effects observed in the clinical trials seem to be minimal.

It was observed that oral administration of Silybum *marianum* ethanolic extract to rats did not adversely affect the body weight and feed efficiency ratio. Similarly, Das and Vasudevan (2006) concluded that silymarin which derived from *Silybum marianum* plant has no effect on body weight of rats when given in a dose of 1.6 g/kg b.wt./day for 4 weeks.

In the present study, oral administration of *Silybum marianum* (SM) extract for 8 weeks to CCL4 -intoxicated rats offered a protection to the liver as evidenced by the histopathological examination of liver sections of the pretreated which showed normal architecture of hepatic lobules. In addition, serum liver enzymes (ALT, AST and ALP) in pretreated rats were normalized when compared to the control CCL4-

intoxicated group. These findings were consistent with those previously recorded by Kim *et al.* (2009) who concluded that the ACTIV aloe complex (mixture of *Aloe vera* and *Silybum marianum*) has a hepatoprotective effect in both acute and chronic liver injuries induced by CCL4. The authors concluded that *Silybum marianum* plant got a bright reputation in relieving the liver diseases that might be due to potent silymarin mixture and its mechanism of action is mainly due to antiradicals scavenging activity. Shaker *et al.* (2010) also found that the ethanolic extract of *S. marianum* significantly decreased the elevated liver enzymes caused by CCL4. These biochemical changes were accompanied by an improvement in histopathogical lesions caused by CCL4 in the pretreated rats with the SM extract.

The antioxidant activity of *Silybum marianum* ethanolic extract was proved by significant increases in the levels of antioxidant enzymes (SOD, GPx and CAT) in liver homogenate in rats. In this concern, Muriel and Mourelle (1990) and Lawrence *et al.* (2000) reported that the flavonoids of *Silybum marianum* had a potent antioxidant effect as indicated by significant decreases of superoxide anions and free lipid and oxygen radicals due to inhibition of lipid peroxidation. Muriel and Mourelle (1990) mentioned that *Silybum marianum* interacts directly with the cell membrane components to prevent any abnormalities in the content of lipid fraction, which is responsible for maintaining normal fluidity. Previous studies reported the potent *in vivo* antioxidant activity of *Silybium marianum* (Murray, 1995 and Shaker *et al.*, 2010). The later author attributed the *in vivo* antioxidant activity of *Silybium marianum* to the increase in the level of reduced glutathione, which is an important antioxidant that detoxifies drugs and chemicals. In addition, Pradhan and Girish (2006) reported that *Silybum marianum* extract produces glutathione enhancer and liver regenerator effects. These authors attributed the protective effect of *silybum marianum* extract against hepatotoxicity induced by carbon tetrachloride are due to the antioxidant properties of flavonoids which present in the plant.

The antioxidant properties of many herbs and spices that were reported to be effective in retarding and inhibiting the process of lipid peroxidation (Namiki, 1990; Pokorny, 1991 and Duh and Yen, 1997). Concerning the *in vitro* antioxidant activity of *Silybum marianum*, it was found the plant extract had a high free radicals scavenger activity that evident by increased inhibition of reaction rate with DPPH radical, in a concentration- dependant manner. However, the extract activity was less potent than that of ascorbic acid. In this concern, Muriel and Mourelle (1990) and Lawrence *et al.* (2000) reported that flavonoids of *Silybum marianum* had a potent antioxidant effect due to scavenging of free radicals, superoxide anions, and oxygen radicals. Moreover, many previous investigations proved the potent *in vitro* antioxidant activity of *Silybum marianum* plant extract such as those reported by Murray (1995); Varga *et al.* (2004); Katiyar (2005) and Saller *et al.* (2007).

In conclusion, *Silybum marianum* has high safety, causes no adverse effect body weight and produces hepatoprotective and antioxidant effects in CCL4 – poisoned rats. The hepatoprotective activity of *Silybum marianum* could be attributed to its antioxidant effect due its ability for free radical scavenger activity. Inhibition of reaction rate using DPPH radical *in vitro* by *Silybum marianum* extract confirms its potent antioxidant activity. Therefore, this study recommends that intake of *Silybium marianum* plant may be useful for patients who suffer from liver diseases associated with oxidative stress.

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References:

Aghazadeh, S.; Amini, R.; Yazdanparast, R. and Ghaffari, S.H. (2011): Anti-apoptotic and anti-inflammatory effects of *Silybum marianum* in treatment of experimental steatohepatitis. Exp. Toxicol. Pathol., 63(3): 569-574.

Badary, O.A.; Taha, R.A.; Gamal El-Din, A.M. and Abdel-Wahab, M.H. (2003): Thymoquinone from *Nigella sativa* seeds is a potent superoxide anion scavenger. Drug Chem. Toxicol., 26(2): 87-98.

Bruck, R.; Hershkoviz, R.O.; Lider, H.; Aeed, L. ; Zaidel, Z.; Matas, J.; Barg, J. and Halpern, Z. (1996): Inhibition of experimentally induced liver cirrhosis in rats by a non peptic mimetic of extracellular matrix of associated Arg-Gly-Asp epitope. J. Hepatol., 24: 731-738.

Carleton, H. (1976): Carleton's Histological technique. 4th Edition, London, Oxford University press New York, Toronto.

Chapman, D.G.; Castilla, R. and Campbell, J. A. (1959): Evaluation of protein in food. I. A method for the determination of protein efficiency ratio. Canadian J. Bioch. Physiol., 37: 679686.

Das, S.K. and Vasudevan, D.M. (2006): Protective effects of silymarin, a milk thistle (*Silybium marianum*) derivative on ethanol-induced oxidative stress in liver. Indian J. Biochem. Biophys., 43: 306-311.

Duh, P.D. and Yen, G.C. (1997): Antioxidant efficacy of methanolic extracts of pea nut hulls in soy beans and

pea nut oils. J. Am. Oil Chem. Soc., 74:745 – 748.

Gad, C.S. and Weil, S.C. (1982): Cited in: Hays, A.W. (Ed.), Principles and Methods of Toxicology, second Edition, Raven Press, NY, USA, page 292 – 294.

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, K.S., Mehrota, S. and Pushpanadan, P. (2003): Studies on the antioxidant activity of *Demodium gangeticum.* Biol. Pharmaceut. Bulletin, 26 (10): 1424-1427.

Hu, Y.Y.; Liu, C.; Xu, L.M.; Liu, C.H.; Zhu, D.Y. and Huang, M.F. (1997): Actions of salvianolic acid on CCL4- poisoned liver injury and fibrosis in rats. Zhanggo Yaoli Xucbao, 18: 478-480.

Kind, P.R.N. and King, E.J. (1954): Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. J. Clin. Pathol., 7: 322-326.

Kim, S.H.; Cheon, H.J.; Yun, N.; Shin, E.; Shim, K.S. and Lee, S.M. (2009): Protective effect o a mixture of A*loe vera* and *Silybum marianum* against carbon tetrachloride-induced acute hepatotoxicity and liver fibrosis. J. Pharmacol. Sci., 109: 119-127.

Katiyar, S.K. (2005): Silymarin and skin cancer prevention: anti-inflammatory, antioxidant and immunomodulatory effects. Int. J. Oncol**.,** 26: 169-176.

Lawrence, V.; Jacobs, B. and Dennehy, C. (2000): Milk Thistle: Effects on liver disease and cirrhosis and clinical adverse effects. Agency for Healthcare Research and Quality, Rockville, CA,USA.

Mourelle, M.; Muriel, P.; Favari, L. and Franco, T. (1989): Prevention of CCL4 – induced liver cirrhosis by silymarin, Fundam. Clin. Pharmacol., 3:183-191.

Muriel, P. and Mourelle, M. (1990): Prevention by silymarin of membrane alterations in acute CCL4 - induced liver damage. J. Appl. Toxicol., 10 (4): 275-279.

Murry, M. (1995): The healing power of herbs. Second Edition, Rockville, CA, USA.

Nadeem, M.C.; Dandiya, K.V.; Pasha, M.; Omran, D.K.; Balani, S. and Vohora, B. (1996): Hepatoprotective effect of *Solanium negrum* fruits. Fetoterapia, 68(3): 245-251.

Namiki, M., (1990): Antioxidants /antimutagenics in Food. Critical Reviews in Food Science and Nutrition, 39: 273-300.

Nishikimi, M.; Rao, N.A. and Yogi, K. (1972): Colorimetric determination of superoxide dismutase in tissues. Biochem. Biophys. Res. Common., 46: 849-854.

Peiwu, L.; Hopia, A.; Jari, S.; Yrjonen, T. and Vuorela, H. (1999): TLC method for evaluation of free radical scavenging activity of rapeseed meal by video scanning technology. The 10th International Rapeseed Congress, Canberra, Australia.

Paglia, D.E. and Valentine, W.N. (1967): Determination of glutathione peroxidase in tissue by UV method. J. Lab. Clin. Med., 70:158-169.

Parola,P.; Leonarduzzi, G.; Biasi, F.; Albano, E.; Biocca, M.E.; Poli, G. and Dianzani, M.U. (1992): Vitamin E supplementation protects against CCl4- induced chronic damage and cirrhosis. Hepatol., 16:1014-1021.

Pradhan, S.C. and Girish, C. (2006): Hepatoprotective herbal drug silymarin from *Silybum marianum*. Ind. J. Med. Res., 124 (5): 491-504.

Pokorny, J. (1991): Natural antioxidants for food use. Trends Food Sci. Technol., 9: 223 – 227.

Post-White. J.; Ladas, E.J. and Kelly, K.M. (2007): Advances in the use of milk thistle (*Silybum marianum*). Integr. Cancer Therp., 6(2): 104-109.

Ramasamy K., and Agarwal, R. (2008): Multitargeted therapy of cancer by silymarin. Cancer Letters, 269: 352-362.

Reeves, P.G.; Nielson, F.H. and Fahmy, G.C.(1993): Reports of the American Institute of Nutrition, Adhoc Willing Committee on Reformulation of the AIN 93, Rodent diet. J. Nutri., 123:1939-1951.

Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28:56-63.

Shalaby, M.A. and Hamowieh, A.R. (2010): Safety and efficacy of *Zingiber officinale* roots on fertility of male diabetic rats. Food Chem Toxicol.; 48:2920-2924.

Sinha, K.A. (1972): Colorimetric assay of catalase enzyme. Anal. Biochem., 47: 389-394.

Snedecor, G.W. and Cocharn, W.G. (1986): Statistical methods, 4th Edition, Iowa State Collage Press, Ames, Iowa, USA.

Shaker, E.; Mahmoud, H. and Mnaa, S.(2010): Silymarin the antioxidant component and *Silybum marianum* prevents liver damage. Food Chem. Toxicol., 48 (3): 803-806.

Saller, R.; Meltzer, J.; Reichling, J.; Brignoli, R. and Meier, R. (2007): An updated systematic review of the pharmacology of silymarin. Forsch. Komplement Med., 14 (2): 70-80.

Tamayo, C. and Diamond, S. (2007): Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum). Integr. Cancer Therp., 6(2): 146-157.

Varga, Z.; Ujhelyi, L.; Kiss, A., Balla, J. and Czompa.A. and Antus, S. (2004): Effect of silybin on phorbol myristate acetate-induced protein kinase C translocation, NADPH oxidase activity and apoptosis in human neutrophils. Phyomed., 11: 206-212.