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ORIGINAL ARTICLE

Hepatoprotective effect of *Silybum marianum* on experimental hepatotoxicity in broilers

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Abstract Uncontrolled lipid oxidation plays a key role in poultry diseases. *Silybum marianum* have been used as a natural medication for the liver and biliary duct. Pharmacologically effective substance of silymarin includes four main ingredients: silybin, silychristin, silydianin, and isosilybin. CCL4, a hepatotoxic chemical agent, is largely used to produce experimental models to study hepatic cirrhosis and fibrosis in experimental animals. The aim of the present study was to verify the hepatoprotective effects of feed mixtures containing *S. marianum* in CCL4-induced hepatotoxicity in broilers. A randomized experimental design was used, and chicks were divided into six treatment groups. Chicks in group 1 (control) were fed basal diets without any supplementation. Birds in group 2 were fed basal diets and received CCL4. Birds in group 3 were fed basal diets supplemented with 60 ppm extract of *S. marianum* fruit (SE). Birds in groups 4 to 6 fed basal diet plus 40 ppm SE+CCL4, 60 ppm SE+CCL4, and 80 ppm SE+CCL4, respectively. Blood samples were analyzed to determine the contents of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein, albumin, globulin, and liver cytochrome P450. In CCL4-treated groups, the mean serum levels of ALT, AST, SGOT, and SGPT were markedly ($P<0.05$) raised compared to the control group, and addition of SE reduced ($P<0.05$) the levels of these enzymes. Liver cytochrome P450 increased with CCL4 and reduced ($P<0.05$) with the coadministration of different concentrations of SE ($P<0.05$). It was observed that treatment with *S. marianum* fruit extract significantly reduced the tissue damage caused by CCL4.

Keywords Silybum marianum · CCL4 · Alanine aminotransferase · Glutamic pyruvic transaminase · Hepatotoxicity

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Introduction

Interactions between nutritional and environmental factors can affect metabolic systems of stocks such as nutritional deficiencies that can cause changes of the antioxidant system. This system is an important scavenger of lipid peroxides and free radicals produced throughout metabolic pathways, division, and differentiation, also during hormone and prostaglandin biosynthesis (Mezes et al. 1997). The organisms which exposed to lipid peroxides in feed absorbed them in the form of unsaturated chetocompounds and initiated tissue lipid peroxidation. Uncontrolled lipid oxidation plays a key role in poultry diseases and toxicosis and affects egg and meat quality.

Studies have been conducted in order to test natural compounds that are capable of improving lipid stability in broilers. It has been demonstrated that dietary supplementation with both synthetic and natural antioxidants like vitamin E, ascorbic acid, selenium, rosemary, and sage extracts can improve antioxidant defenses (Carreras et al. 2004; Young et al. 2003).

Silybum marianum have been used as a natural medication for the liver and biliary duct since ancient times. Pharmacologically effective substance of silymarin includes four main ingredients: silybin, silychristin, silydianin, and isosilybin (Ding et al. 2001). Silymarin has various activities, antioxidant activity, intracellular glutathione regulator; stabilizer, and cell membrane permeability regulator, that

limits the entry of toxic factors into liver cells; as promoter of ribosomal RNA synthesis, it causes regeneration of the hepatic damages and inhibits the formation of myofibroblasts from stellate cells. Furthermore, absorption of free radicals is considered to be one of the key mechanisms securing liver protection (Fraschini et al. 2002).

Although silymarin has commonly been used in human clinical practice for quite a long time, some mechanisms of its hepatoprotective effect have not been discovered yet. Crocenzi and Roma (2006) point out its potent anticholestatic activity in their review of clinical studies regarding the hepatoprotective effect of *silymarin*. It is suggested that silymarin can directly impact on the cholesterol metabolism by means of inhibiting its biosynthesis (Škottová and Krečman 1998).

Many scientists point out the anticancer potential of silymarin as it is able to suppress the proliferation of tumor cells (the prostate, breast, ovary, colon, lung, and bladder) (Singh and Agarwal 2004; Agarwal et al. 2006). The silymarin has protective effects on pancreatic damage induced by alloxan (Soto et al. 2003), and also, it reduced hyperglycemia and induced reversion of the pancreatic damage in alloxan-treated rats (Soto et al. 2004).

The effects of silymarin or various products made from the seed of the plant *S. marianum* containing silymarin have also been tested on farm animals. The hepatoprotective activities were assessed on the basis of selected biochemical blood indicators in chicken broilers that had been exposed to the effects of aflatoxins (Tedesco et al. 2004). The protective activities of silymarin on the liver were observed in aflatoxin B1 application on rats (Rastogi et al. 2000). The effectiveness of silymarin was also confirmed in dogs and cats when administering large doses of oxytetracycline which has a hepatotoxic effect (Vijayakumar et al. 2004). Many scientists have focused on the effects of products made from *S. marianum* seeds containing silymarin regarding utility indicators in chicken broilers (Gawel et al. 2003; Mekala et al. 2006).

Among chemical agents, CCL4, a hepatotoxic chemical agent, is largely used to produce an experimental model to study hepatic cirrhosis and fibrosis in experimental animals (Taniguchi et al. 1990). Hepatotoxic effects of CCL4 by metabolic activation are well established; therefore, it induces toxicity in hepatic cells maintaining seminormal metabolic function (Mujumddar et al. 1998). Exposure to CCL4 results in hepatic steatosis, centrilobular necrosis, and ultimately cirrhosis in the liver and acute tubular necrosis in the kidney (Karmia Moawad 2007). Hepatoprotection by conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious side effects (Guntupalli et al. 2006). In the absence of an effective liver-protective drug in modern medicine, there are a number of medicinal plants that is recommended for the treatment of liver disorders (Chatterjee 2000). In view of severe undesirable side effects of synthetic agents, there is an emerging focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that has protective effects on the liver.

The aim of the present study was to verify the hepatoprotective effects of feed mixtures containing the *S. marianum* in CCL4-induced hepatotoxicity in broilers.

Materials and methods

A completely randomized experimental design was used, and chicks were divided into six treatment groups, with four replicates per treatment and 12 chicks per replicate. A total of 288, 1-day-old broiler chicks (Ross 308) were raised in floor pens with ad libitum access to feed and water and controlled ventilation. Chicks of the same body weight, a mean initial body weight of 50 g, were placed in pens. Temperature was maintained at 32 °C for the first 4 days and then gradually reduced according to normal management practices until a temperature of 22 °C was achieved at day 28. The light program was 23 h of light and 1 h of dark. Basal diet was formulated according to NRC (1994) for broilers from 0 to 6 weeks of age (Table 1). During the

Table 1 Composition of the basal diet in the experiment (NRC 1994)

Item	0–3 weeks	4–6 weeks
Ingredient (%)		
Ground yellow maize	56.6	60.0
Soybean meal	36	32
Maize oil	3	3.5
Dicalcium phosphate	1.8	1.9
Limestone	1.3	1.3
NaCl	0.3	0.3
DL-Met	0.1	0.1
Choline chloride	0.14	0.14
Premix[a]	1	1
Nutrient level		
ME (MJ/kg)	12.54	12.85
CP (%)	20.94	19.80
Lys (%)	1.20	1.05
Met (%) 0.54	0.54	0.47
Cys (%)	0.36	0.28
Ca (%) 1.05	1.05	1.00
Available P (%)	0.51	0.45

[a] Supplied per kilogram of diet as follows: Cu 10 mg, Fe 90 mg, Mn 90 mg, Zn 50 mg, Se 0.2 mg, I 0.4 mg, Co 0.4 mg, vitamin A 5,000 IU, cholecalciferol 500 IU, vitamin E 10 IU, riboflavin 6.0 mg, pantothenic acid 12 mg, niacin 35 mg, cobalamin 10 μg, biotin 0.8 mg, folic acid 0.8 mg, thiamine 1.5 mg, and pyridoxine 1.5 mg

experiment, no antibiotics were given to broilers. The study had a 6-week duration.

Animal design

Chicks in group 1 (control) were fed basal diets without any supplementation. Birds in group 2 were fed basal diets and received CCL4 (1 ml/kg of body weight orally). Birds in group 3 were fed basal diets supplemented with 60 ppm extract of *S. marianum* fruit (SE), (PLUSIL; BIOTRADE snc, Italy). Birds in groups 4 to 6 were fed basal diet plus 40 ppm SE+CCL4 (1 ml/kg), 60 ppm SE+CCL4 (1 ml/kg), and 80 ppm SE+CCL4 (1 ml/kg), respectively.

Sampling

Blood samples of five birds per replicate (selected randomly) were collected from the wing vein. Blood in EDTA-containing tubes was collected for determination of blood profile, while whole blood samples for determination of serum proteins were collected without anticoagulant. Serum was separated after centrifugation of clotted whole blood at 3,500 rpm for 20 min. Serum and EDTA-containing blood were kept at 4 °C for further analysis. Blood samples were analyzed by Vet Med Labor GmbH (Ludwigsburg 71611, Germany) to determine the contents of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein, albumin, and globulin.

Determination of liver microsomal cytochrome P450

In order to determine the levels of liver microsomal cytochrome P450, the liver microsomal fractions were prepared according to Latif (2010). The pellet of the second centrifugation was resuspended in 0.25 M sucrose containing 10 mM phosphate buffer, pH 7.4, and was used for the determination of microsomal cytochrome P450. The concentration of lung and liver cytochrome P450 homogenates was estimated from the dithionite-reduced difference spectrum of CO-bubbled samples, using an absorption coefficient of 91 mM^{-1} cm^{-1}. Aliquots of tissue homogenate and microsomal pellets from each subfraction were analyzed for protein concentration using the BCA Protein Assay (Latif et al. 2010).

Statistical analysis

When the chicks reached 42 days of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (SAS Institute 2002). The treatment means with significant differences were compared by using Tukey's new multiple range tests. All statements of differences were based on significance at $P<0.05$.

Results

Biochemical parameters

ALT and AST levels

According to Table 2, the mean serum levels of ALT and AST in CCL4-treated groups show a marked raise compared to the control ($P>0.001$ and $P=0.023$, respectively). Simultaneous administration of SE (40, 60, and 80 ppm) and CCL4 reduced the serum levels of ALT and AST

Table 2 Effects of different doses of silymarin on serum ALT and AST levels

Group	Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	ALT (U/I)	7.3±0.7	7.4±0.5a	6.9±0.6a	7.7±0.9ab	8.1±0.8ab	7.8±0.6b	8.0±0.9b
	AST (U/I)	191±23	188±31ab	185±34a	193±27b	190±37b	195±41b	189±35b
2	ALT (U/I)	7.1±0.6	9.3±1.1c	9.8±1.2d	9.7±0.9c	10.6±1.3c	12.2±1.6d	12.1±1.5d
	AST (U/I)	194±33	209±42c	230±48c	248±39c	266±44d	293±49d	304±45d
3	ALT (U/I)	7.7±0.7	7.3±0.5a	7.0±0.8a	6.8±0.4a	7.1±0.8a	6.6±0.5a	6.7±0.4a
	AST (U/I)	186±13	180±21a	182±17a	177±22a	179±14a	175±17a	170±19a
4	ALT (U/I)	7.4±0.6	8.0±0.7ab	8.3±0.6ab	8.4±0.8b	9.0±1.1bc	10.1±1.3c	10.8±1.1c
	AST (U/I)	193±22	200±24b	204±21c	211±22b	225±31c	249±27c	263±33c
5	ALT (U/I)	7.0±0.7	7.8±0.6ab	7.9±0.9ab	8.2±1.2b	8.6±0.9b	8.5±0.7b	9.2±1.1bc
	AST (U/I)	195±19	198±21b	205±24c	208±28b	216±26c	223±28bc	237±29c
6	ALT (U/I)	7.8±0.6	7.6±0.6a	8.2±0.9ab	8.4±0.7b	7.9±0.9ab	8.0±1.1b	7.9±0.8b
	AST (U/I)	192±26	195±24b	195±25b	203±29b	209±32c	214±34bc	213±28bc

Letters a-c show significant differences in any column ($P<0.05$ and $P<0.01$)

ALT alanine aminotransferase, *AST* aspartate aminotransferase

Table 3 Effects of different doses of silymarin on serum SGOT and SGPT levels

Group	Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	SGOT (U/I)	254±34	251±37a	253±29a	256±31a	252±41a	248±33a	257±35b
	SGPT (U/I)	22±3	23±5a	22±2a	21±4a	24±3ab	23±4b	22±3a
2	SGOT (U/I)	258±28	267±26c	270±32c	274±37c	273±31c	277±41d	284±42d
	SGPT (U/I)	21±4	25±5cb	28±4b	33±2c	34±5c	37±5c	43±3c
3	SGOT (U/I)	259±32	256±33b	255±28a	258 ±33a	260±34b	252±29b	248±31a
	SGPT (U/I)	20±3	22±5a	21±3a	19±2a	22±4a	19±3a	20±3a
4	SGOT (U/I)	262±26	268±28c	269±31c	273 ±28c	272±24c	273±32d	276 ±29c
	SGPT (U/I)	23±4	25±5b	27±3b	31±6c	31±6c	32±7c	40±6c
5	SGOT (U/I)	257±33	260±25bc	264±32bc	268±28bc	266±31bc	266±32c	272±29c
	SGPT (U/I)	22±3	21±4a	23±5a	25±3b	25±6b	28±4bc	32±5b
6	SGOT (U/I)	251±19	253±33a	262±34b	264±26b	262±29b	258±31b	262±34b
	SGPT (U/I)	20±3	22±4a	21±3a	23±5ab	24±3ab	26±5b	29±4ab

Letters a–d show significant differences in any column ($P<0.05$ and $P<0.01$). *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase

compared to CCL4-treated group ($P>0.038$, $P>0.011$, and $P>0.001$, respectively). The levels of ALT and AST were restored to near normal in CCL4 with 80 ppm SE group ($P>0.048$), whereas in groups 4 and 5 (40 and 60 ppm SE), these levels were higher than normal when compared to control group ($P>0.022$ and $P>0.037$, respectively; Table 2).

SGOT and SGPT

The mean SGOT and SGPT values were found to be elevated significantly ($P<0.01$) in CCL4-intoxicated control group. SE coadministration with CCL4 in groups 4 to 6 shows a significant reduction in SGOT and SGPT levels and best results achieved in group 6 with 80 ppm SE coadministration (Table 3).

Total protein, albumin, and globulin levels

In case of hepatotoxicity or liver damage, hypoproteinemia is an accepted event. However, results of the present study did not show significant changes in total protein, albumin, and globulin levels ($P>0.05$). The regenerative ability of liver and CCL4's non-severe toxic dose could account for these findings (Tables 4 and 5). Nonsignificant changes in total protein levels were observed only in group 2.

Liver cytochrome P450

The liver cytochrome P450 is shown in Table 6. The CCL4 control group showed an increment of liver P450 levels as time advanced. Significant reduction in cytochrome P450 levels were seen in the SE-treated groups 4 to 6 ($P<0.05$).

Table 4 Effect of different doses of silymarin on serum albumin and globulin levels

Group	Parameter	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	Albumin (g/l)	1.24±0.3	1.12±0.3	1.15±0.2	1.27±0.4	1.18±0.3	1.24±0.4	1.22±0.3
	Globulin (g/l)	3.07±0.3	3.10±0.4	2.95±0.3	3.12±0.4	3.07±0.3	3.02±0.4	3.04±0.3
2	Albumin (g/l)	1.12±0.2	1.05±0.1	1.00±0.1	0.99±0.1	0.98±0.1	0.95±0.2	0.91±0.2
	Globulin (g/l)	3.06±0.2	2.94±0.3	2.95±0.3	2.90±0.2	2.85±0.4	2.82±0.3	2.82±0.4
3	Albumin (g/l)	1.14±0.4	1.30±0.3	1.32±0.3	1.31±0.2	1.35±0.3	1.34±0.4	1.35±0.3
	Globulin (g/l)	3.09±0.6	3.10±0.5	3.14±0.4	3.12±0.5	3.18±0.6	3.22±0.3	3.30±0.6
4	Albumin (g/l)	1.20±0.4	1.12±0.3	1.07±0.2	1.02±0.3	1.00±0.2	0.96±0.2	0.98±0.2
	Globulin (g/l)	3.03±0.4	3.00±0.5	2.95±0.5	2.94±0.4	2.95±0.3	2.92±0.4	2.90±0.3
5	Albumin (g/l)	1.18±0.3	1.15±0.2	1.12±0.2	1.07±0.1	1.03±0.2	1.04±0.2	1.04±0.3
	Globulin (g/l)	3.09±0.3	3.02±0.4	3.02±0.5	3.00±0.4	3.00±0.4	2.98±0.4	2.99±0.3
6	Albumin (g/l)	1.19±0.3	1.15±0.5	1.11±0.4	1.10±0.5	1.08±0.4	1.09±0.4	1.10±0.3
	Globulin (g/l)	3.08±0.5	3.05±0.5	3.05±0.6	3.03±0.4	3.04±0.4	3.06±0.5	3.06±0.6

Table 5 Effect of different doses of silymarin on serum total protein (in gram per liter) levels

Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	32±3.5	35±3.4	36±3.2	42±3.3	45±3.5	47±2.9	48±2.3
2	33±3.6	36±3.2	37±3.3	44±3.4	43±3.7	45±3.4	47±3.1
3	32±3.5	33±4.1	39±3.5	40±3.7	46±3.2	45±4.1	49±2.9
4	31±3.7	37±3.3	38±3.6	39±4.1	45±2.7	48±3.5	46±3.3
5	34±3.4	36±3.2	37±3.1	43±2.8	42±4.2	44±2.8	45±3.7
6	34±2.9	35±3.5	40±3.6	44±3.2	46±3.8	46±3.3	47±3.2

The highest reduction in cytochrome P450 levels were seen in group 6 with 80 ppm SE coadministration (P=0.007). SE coadministration with 60 and 80 ppm concentration during days 35 and 42 had the same effects on cytochrome P450 levels.

Discussion

In the present study, it was found that the *S. marianum* can modulate the hepatotoxicity induced by CCL4.

Silymarin is a scavenger of free radicals like superoxide, hydroxyl, and hydrogen peroxide (H_2O_2), which increases sorbitol dehydrogenase and decreases lipid peroxidation (Oliveira et al. 2001; Lappin 2001). It protects hepatic cells via stabilizing the membrane permeability through inhibiting lipid peroxidation (Najafzadeh et al. 2010; Mira et al. 1994) and prevents liver glutathione depletion (Valenzuela and Garrido 1994). Antioxidant property is the major activity of silymarin which makes it effective in the prevention of other organ-specific toxicities related to the induction of oxidative stress (Varzi et al. 2007).

The toxicity produced by CCL4 is mediated through free radical mechanism. The CCL4 is metabolized by cytochrome P450 enzyme, and its metabolic products, trichloromethyl free radicals, are highly reactive and induce lipid peroxidation of macromolecules leading to tissue injury (Taniguchi et al. 1990).

Serum ALT and AST activity was lower for SE-treated groups compared to CCL4 control (P<0.05). ALT and AST activity are sensitive indicators of liver damage (Lumeij 1997; Yousefi et al. 2005).

The activities of SGOT and SGPT are the most commonly used biomarkers of liver damage (Sturtgill and Lambart 1997). The hepatotoxic action of CCL4 is due to its toxic metabolite, trichloromethyl radicals (He et al. 2006; Lee et al. 2007). The SGOT and SGPT values were found to be elevated significantly (P<0.01) in CCL4-intoxicated group 2 as compared to healthy control group A. The results in the present study for group 2 are in concurrence with those reported by Kanter et al. (2005) and Dahiru et al. (2005) who reported a significant increase in SGOT and SGPT in CCL4-induced fatty liver. However, the values of these enzymes were reduced in SE-treated groups, and the values were well comparable to those of healthy control group 1. This indicates efficacy of active ingredients of SE.

Cytochrome P450 3A4 is an important phase I enzyme in xenobiotic metabolism. Several studies have shown that silymarin inhibits CYP450 activity (Kiruthiga et al. 2007). An in vitro study with silybin concentrations of 25, 50, 100, and 250 μM indicated that CYP450 3A4 inhibition depended on silymarin concentration and length of exposure (Sridar et al. 2004).

The present results revealed that the extract could protect the organs from toxicity induced by chemical compounds. This activity can be partially attributed to the free radical scavenging activity and enhancement of the antioxidant system effectively by the extract since many of the active ingredients present in the extract are potent free radical scavengers. However, other mechanism such as its effect on cytochrome P450 enzymes may also be looked into.

Table 6 Effect of different doses of silymarin on liver cytochrome P450 (in nanomole per milligram of protein) levels

Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	0.242±0.026	0.235±0.041a	0.234±.0032a	0.242±0.033a	0.245±0.055a	0.247±0.049a	0.243±0.033a
2	0.248±0.036	0.386±0.032c	0.387±0.033d	0.380±0.034d	0.381±0.037d	0.375±0.034d	0.370±0.031d
3	0.244±0.035	0.233±0.041a	0.239±0.035a	0.240±0.037a	0.246±0.032a	0.245±0.041a	0.249±0.029a
4	0.241±0.037	0.327±0.033b	0.328±0.036c	0.319±0.041c	0.315±0.027c	0.310±0.035c	0.310±0.033c
5	0.248±0.034	0.326±0.032b	0.320±0.031bc	0.310±0.028bc	0.307±0.042bc	0.274±0.028b	0.265±0.037b
6	0.239±0.039	0.335±0.045a	0.340±0.036a	0.304±0.039b	0.296±0.038b	0.276±0.033b	0.277±0.032b

Letters a–d show significant differences in any column (P<0.05 and P<0.01)

In this study, silymarin displayed the hepatoprotective effect suggested by other authors (Erdogan et al. 2005; Lang et al. 1990; Schiavone et al. 2007).

Conclusion

This study showed that silymarin has a significant protection against CCL4-induced hepatotoxicity. The silymarin has been used as a medicine for many years. As there is no significant toxicity in animal studies, this plant extract can be administered as a prophylactic, dietary supplement. It was observed that treatment with *S. marianum* fruit extract significantly reduced the tissue damage caused by CCL4. This is evident from the decreased level of marker enzymes of tissue injury. The results indicate a protective effect of *S. marianum* fruit extract against hepatotoxic agents.

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