

Effect of Water Extract of the Milk Thistle (*Silybum marianum* L.) on Some Liver Enzymes

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Abstract

We examined the effect of water extract from the aboveground part of milk thistle (*Silybum marianum* L.) and pure silymarin on alaninaminotransferase (ALT) (EC 2.6.1.2), aspartataminotransferase (AST) (EC 2.6.1.1), acidic and alkaline phosphatase (EC 3.1.3.3 and EC 3.1.3.1) of livers, *in vitro* and *in vivo* conditions.

Key words: liver, *Silybum marianum* L., ALT, AST, acidic and alkaline phosphatase

Introduction

The milk thistle (*Silybum marianum* L.) is a native herb of Asia Minor, North Africa and southern Europe. It has been acclimatized to central Europe, North and South America, and South Australia. The herb has dark-green prickly leaves mottled or streaked with white veins. It blooms from June until September and can grow up to six feet tall (<http://www.newhope.com/nutritionsciencenews>). Silymarin extracted from milk thistle has shown a protective effect against many types of chemical toxins, including alcohol. The extract of milk thistle is used to improve liver function, protect against liver damage and enhance regeneration of damaged liver cells (<http://www.bodyandfitness.com/products/Health/herbal>).

Recently, this plant was cultivated in Mongolia. Some researchers have studied only the seed chemical composition of milk thistle, but we studied the effects of water extract from the aboveground part and pure silymarin from this plant's seed on alaninaminotransferase (ALT) (EC 2.6.1.2), aspartataminotransferase (AST) (EC 2.6.1.1), acidic and alkaline phosphatase (Ac.P and Al.P) (EC 3.1.3.3 and EC 3.1.3.1) of sheep and horse livers *in vitro* and at rabbit serum *in vivo*.

Material and Methods

We determined the ALT and AST activities

by the dinitrophenylhydrazin method (Purev & Bayarmaa, 2003) (one unit was measured as 1 mg of enzymatic protein could change the light absorption by 0.01 in 1 hour; $\lambda=750\text{nm}$, $d=10\text{mm}$), acidic and alkaline phosphatase activities were determined by the Bodansky method (Purev & Bayarmaa, 2003) (one unit was measured as 1 μg phosphorus amount, which was produced by 1 mg enzymatic protein per minute; $\lambda=750\text{nm}$, $d=10\text{mm}$). Protein content was determined by the Lowry method (Tsevegsuren & Purev, 2001), and the infused substance amount by the dehydration method (Purev, 2006).

Results

First, we examined the protein content of sheep ($12.2\pm 1.1\%$) and horse ($9.4\pm 2.3\%$) livers as well as in fresh weight of milk thistle (Table 1). This result indicates that the protein content of sheep liver is 1.3 times higher than that in the horse liver. We calculated the Standard deviation (1 SD) in each of five trials (Tables 1, 2 and 3).

As shown in Table 1, milk thistle contained approximately $6.5\pm 1.2\%$ protein in fresh weight, but it was shown by other researchers that milk thistle seeds contained 25-30% protein (<http://www.healingdaily.com/liver-detoxification/liver-detoxification-other-herbs.htm>). By their investigations the amount of seed protein was 3.8-4.6 times higher than the amount in the plant's body.

We determined that the ALT, AST, acidic and alkaline phosphatase activities were 1.15-1.69 times higher in horse liver than that in the liver of sheep. Furthermore, the AST activity was 1.88-2.79 times higher than ALT activity, and acidic phosphatase activity was 1.63-1.75 times higher than alkaline phosphatase activity in both horse and sheep livers (Figure 1).

Table 2 describes how water extract of the aboveground part of milk thistle affects the ALT

and AST activities of sheep and horse livers.

Table 3 describes how the water extract of the aboveground part of milk thistle affects the acidic and alkaline phosphatase activities of sheep and horse livers.

The ALT, AST, acidic and alkaline phosphatase activities increase during liver diseases, but was usually decreased by liver drugs (http://www.aidsinfonet.org/factsheet_detail.php). Milk thistle seed preparations have decreased ALT,

Table 1 Protein amount in livers of horse, sheep and in the sample of milk thistle

№	Samples	Protein amount, %
1	Sheep liver	12.2±1.1
2	Horse liver	9.4±2.3
3	Milk thistle	6.5±1.2

Table 2. Effects of the water extract of aboveground part of milk thistle to the ALT, AST activities of sheep and horse livers

Samples	Infused substance amount of plant, µg	Enzyme activity, by unit	
		ALT	AST
Sheep liver	0	101.5±45.9	190.8±33.6
	4.8	128.2±46.1	206.4±21.1
	9.6	123.6±52.8	210.2±25.6
	14.4	122.0±45.9	223.8±19.3
Horse liver	0	102.6±18.1	273.3±106.6
	4.8	116.6±15.0	321.3±134.4
	9.6	123.9±27.2	328.2±134.0
	14.4	117.9±14.6	357.3±165.8

Table 3. Effects of the water extract of aboveground part of milk thistle to the acidic and alkaline phosphatase activities of sheep and horse livers

Samples	Infused substance amount of plant, µg	Enzyme activity, by unit	
		Acidic Phosphatase	Alkaline Phosphatase
Sheep liver	0	88.8±6.7	52.4±8.0
	9.6	98.6±6.0	54.8±6.9
	19.2	99.3±6.2	54.9±6.4
	28.8	104.4±11.0	57.4±7.5
Horse liver	0	138.2±18.9	78.8±12.0
	9.6	145.3±14.4	84.9±9.3
	19.2	143.9±12.7	86.6±11.5
	28.8	153.5±20.4	95.2±11.7

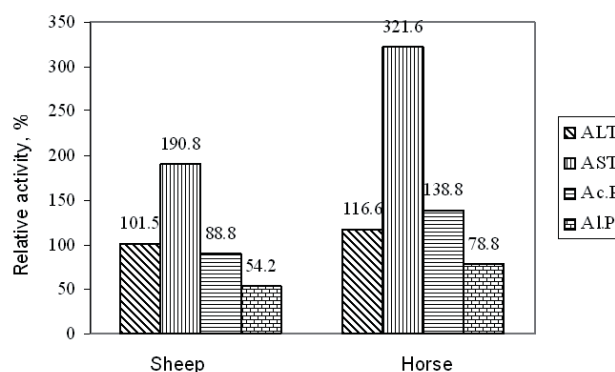


Figure 1. Comparison of activities of ALT, AST, acidic and alkaline phosphatases in sheep and horse livers.

AST, acidic and alkaline phosphatase activities in other studies (<http://www.liversupport.com>). But in our study it showed an opposite result, so we performed a study to confirm this result. We used a drug preparation containing pure silymarin from milk thistle seed. Results of the study are described in Figures 2 and 3.

In water extracts of sheep and horse livers the ALT and AST activities were decreased by 6.8-10.4% and 5.4-7.4%, respectively, and acidic and alkaline phosphatase activities were decreased by 5.5-10.8% and 18.4-21.6%, respectively by pure silymarin. The liver drug Carsil showed reduction of liver ALT, AST, acidic and alkaline phosphatase activities, but the water extract of the aboveground part of milk thistle increased the ALT, AST, acidic and alkaline phosphatase activities. It was necessary to confirm the results of the above experiments on live animals (rabbits). Before the study, a rabbit blood sample was collected in a centrifuge tube. Prepared serum from the blood was assayed and stored. These samples were used

as a control. A working solution from the water extract of the aboveground part of milk thistle (48 μ g of infused substance) and Carsil drug silymarin (0.4mg of infused substance) were prepared, and 10ml of each solution was given to the rabbits. After 3 hours we took blood samples from each rabbit and prepared serum from the blood, and determined ALT, AST, acidic and alkaline phosphatase activities. Results are shown in Figures 4 and 5.

In water extracts of sheep and horse livers, ALT, AST, acidic and alkaline phosphatase activities were increased by 1.06-1.31 times higher *in vitro* when using the water extract of the aboveground part of milk thistle. However, in rabbit serum it decreased the ALT, AST, acidic and alkaline phosphatase activities by 1.15-1.76 times *in vivo*. ALT activity was 1.17 times higher than AST activity and acidic phosphatase activity was 1.79 times higher than alkaline phosphatase activity in rabbit serum, which drank the water extract of aboveground part of milk thistle. This result

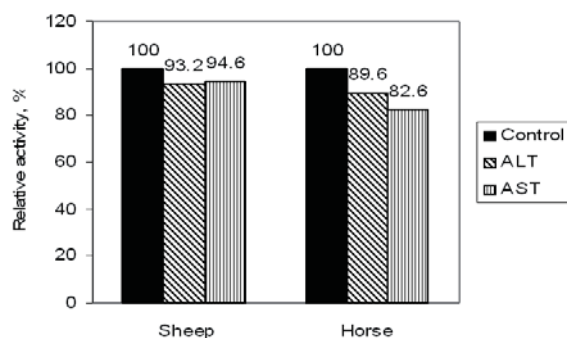


Figure 2. Effect of the silymarin to the ALT, AST activities of sheep and horse livers (silymarin amount is 80 μ g)

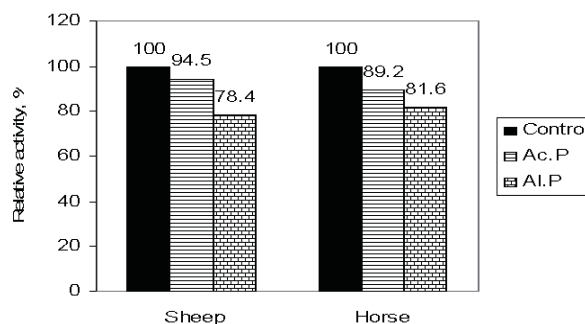


Figure 3. Effect of the silymarin to the acidic and alkaline phosphatase activities of sheep and horse livers (silymarin amount is 80 μ g)

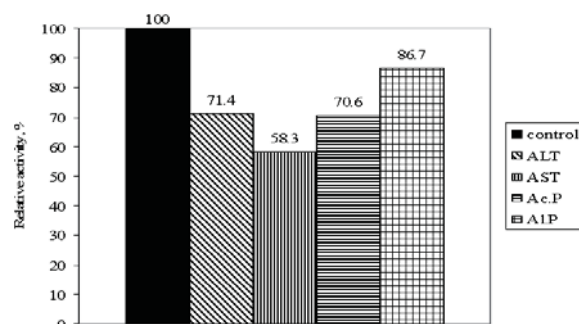


Figure 4. Effect of the water extract of aboveground part of milk thistle on the rabbit serum enzyme activities *in vivo*.

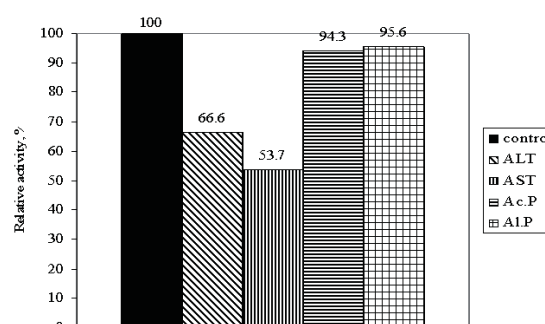


Figure 5. Effect of the water extract of Carsil drug on the rabbit serum enzyme activities *in vivo*.

indicates that the water extract of the aboveground part of milk thistle can decrease the AST activity by 41.7% and the alkaline phosphatase activity by 13.3%.

Carsil drug can decrease liver ALT, AST, acidic and alkaline phosphatase activities by 1.05-1.5 times higher *in vitro*, and by 1.05-1.86 times higher *in vivo*. In the blood serum of rabbits that drank Carsil drug ALT activity was 1.29 times higher than that of AST, and the acidic phosphatase activity was 1.24 times higher than alkaline phosphatase activity. Carsil drug can decrease AST activity by 33.4% at best and alkaline phosphatase activity by 4.4% at worst compared to the water extract of the aboveground part of milk thistle. These results indicate that both the water extract of aboveground part of milk thistle and Carsil drug can decrease rabbit serum ALT, AST, acidic and alkaline phosphatase activities *in vivo*. The water extract of aboveground part of milk thistle can decrease rabbit serum ALT, AST, acidic and alkaline phosphatase activities 1.1-1.4 times more than silymarin solution, but silymarin solution can decrease ALT activity 1.33 times more than the plant extract.

A drug named Silodin produced by our scientist (Ligaa et al, 2006) decreased ALT and AST activities of human serum 2.4 and 2.7 times, thus 1.74 and 1.53 times higher than the water extract of aboveground part of milk thistle, and 1.53 and 1.8 times higher than Carsil drug. This could be because Silodin drug contains four different plant extracts (Ligaa et al, 2006). Also it might be because our plant extraction only contains little amount of silymarin.

Conclusion

We attained the following conclusions. The water extract of aboveground part of milk thistle increased ALT, AST, acidic and alkaline phosphatase activities of sheep and horse livers *in vitro* (1.06-1.31 times). This result indicated that effect of silymarin was inhibited by other compounds in the plant.

Carsil drug decreased ALT, AST, acidic and alkaline phosphatase activities of sheep and horse livers *in vitro* (1.05-1.5 times), showing the effect of pure silymarin.

The water extract of the aboveground part of milk thistle decreased rabbit serum ALT, AST, acidic and alkaline phosphatase activities

in vivo (1.15-1.76 times). Probably because the organism neutralizes the other inhibitor compounds of silymarin.

Carsil drug decreased ALT, AST, acidic and alkaline phosphatase activities of rabbit serum *in vivo* (1.05-1.86 times).

Silymarin influenced AST the most and alkaline phosphatase the least.

In the future, we need to determine the reason and mechanism why the water extract of the aboveground part of milk thistle increased ALT, AST, acidic and alkaline phosphatase activities of sheep and horse livers *in vitro*, but decreased those enzyme activities *in vivo*.

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Хураангуй

Элэгний өвчний улмаас нэмэгддэг аланинаминотрансфераза (АлаТ) (ЕС 2.6.1.2), аспаргатаминотрансфераза (АсаТ) (ЕС 2.6.1.1), хүчиллэг болон шүлтлэг фосфатаза (ХФ ба ШФ) (ЕС 3.1.3.3 ба ЕС 3.1.3.1) ферментүүдийн идэвхийг бууруулахад нь эмчилгээний гол үзүүлэлт болдог. Энэ зорилгоор толбот арзаахай (*Silybum marianum* L.)-ыг хэрэглэдэг. Бидний судалгаанд толбот арзаахайн үрнээс ялгасан силимарин бүхий карсил эмийн бэлдмэл *in vitro* нөхцөлд хонь болон адууны элэгний АлаТ, АсаТ, ХФ, ШФ-ын идэвхийг бууруулж (1.05-1.5 дахин) байхад,

толбот арзаахайн газрын дээрх хэсгийн усан ханд *in vitro* нөхцөлд хонь болон адууны элэгний АлаТ, АсаТ, ХФ, ШФ-ын идэвхийг нэмэгдүүлснийг (1.06-1.31 дахин) силимарины үйлчлэлийг ургамалд хамтдаа агуулагдах өөр нэгдэл саатуулж байна гэж үзлээ.

In vivo нөхцөлд карсил болон толбот

арзаахайн газрын дээрх хэсгийн усан ханд туулайн цусны ийлдсэнд АлаТ, АсаТ, ХФ, ШФ-ын идэвхийг бууруулсныг (тус бүр 1.05-1.86 ба 1.15-1.76 дахин) тус ханданд агуулагдах силимарины үйлчлэлд саад болдог бодисыг организм саармагжуулдагаар тайлбарлаж болох юм гэж үзэж байна.

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