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## Effects of Silymarin and Food Restriction on Hepatic and Pancreatic Functions in Wistar Rats

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**Abstract:** Silymarin, a natural hepatoprotector, extracts from *Silybum marianum*. It seems that silymarin can modulate metabolic changes in the liver and pancreas, also Food Restriction (FR) affects their metabolism. The aim of the study was the evaluation of concurrent effects of silymarin and food restriction on metabolic functions of liver and pancreas to improve their treatment response. Sixty female Wistar rats were divided into six equal groups. Control group were fed ad libitum (Non-FR). Rats in other groups received 50% of the food intake of Non-FR group and served as FR groups. Three of five FR groups received 100, 200 and 400 mg kg<sup>-1</sup> silymarin, respectively. The fourth FR group received placebo. The last FR group and the Non-FR group did not receive any silymarin. Food restriction decreased serum triglycerides in all FR groups (p<0.01). Administration of 100 mg kg<sup>-1</sup> of silymarin to FR rats increased serum triglycerides levels (p = 0.02). Food restriction significantly decreased ALT and ALP which may relate to decrease in liver functional mass. The compare of serum amylase levels in FR and Non-FR groups showed that food restriction, except in FR-100 group, decreased these levels (p<0.05). Present results showed no constant change on glucose and triglyceride levels by silymarin. It seems that silymarin is a modulator of metabolic factors, such as glucose and triglyceride, which needs further researches. Also, concurrent use of food restriction and silymarin doesn't have any adverse effect on pancreas and hepatobiliary system and can be used simultaneously to increase their benefits.

**Key words:** Silymarin, food restriction, liver, pancreas, rat

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### INTRODUCTION

Silymarin as a natural hepatoprotector is a standardized extract from the ripe seeds of *Silybum marianum* (Milk Thistle). Silymarin is a mixture of flavonolignanes in which silibinin is the main compound. Although, silymarin performs its properties in different ways, a range of ones has been related to its antioxidant activities (Fraschini *et al.*, 2002; Saller *et al.*, 2007).

Silibinin and silymarin have important and protective activities against oxidative stress on various organs, such as liver, pancreas and gastrointestinal tract (Fraschini *et al.*, 2002; Soto *et al.*, 1998, 2003, 2004; Kren and Walterov, 2005).

Liver and gastrointestinal tract are responsible for 25% of resting energy expenditure in the animals (Ramsey *et al.*, 2000). These are the first tissues that lose the masses after

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starvation. Changes in the masses of liver and gastrointestinal tract are in direct proportion to dietary intake (Ramsey *et al.*, 2000). It suggested that the observed decrease in total energy expenditure, during food restriction was not only due to the loss of metabolically active tissues, but also because of the decrease in the metabolic rate of the remaining tissues (Even and Nicolaidis, 1993).

Caloric restriction reduces fat mass, serum triglycerides, lipid biosynthesis as well as metabolism in the animals (Skottova and Krecman, 1998; Fan *et al.*, 2003; Spindler *et al.*, 2003). It has been found that silymarin and silibinin may diminish triglyceride synthesis in the liver (Skottova and Krecman, 1998).

Protein synthesis and turnover increase in caloric restricted rodents (Gredilla and Barja, 2005). Also, the expression of some gluconeogenic enzymes increases in caloric restriction (Spindler *et al.*, 2003). In addition, it has been suggested that silymarin could stimulate protein synthesis in the injured liver (Fraschini *et al.*, 2002).

The liver plays a critical role in maintaining glucose homeostasis (Spindler *et al.*, 2003). Caloric restriction reduces blood glucose and insulin concentrations in rodents (Spindler *et al.*, 2003). It has been observed that silymarin is able to prevent the rise in both plasma glucose and pancreatic lipid peroxidation in the hyperglycemic rats (Soto *et al.*, 1998; Kren and Walterov, 2005).

As mentioned above, silymarin and caloric restriction have beneficial effects on liver and pancreatic performance. The aim of the present study was to evaluate the concurrent effects of silymarin and food restriction on metabolic functions of liver and pancreas in order to improve their treatment response.

## MATERIALS AND METHODS

Silymarin extract (82% purity and minimum 30% silibinin) was from Sinochem Qingdao Co., Ltd. (China).

### Animals and Housing Conditions

The laboratory animals were treated in compliance with the Guide to the Care and Use of Experimental Animals (Olfert *et al.*, 1993). The experiment was conducted in 2006-2007 at Islamic Azad University-Kazerun Branch.

Sixty female Wistar rats, 6 months age, were prepared from animal house of Research Centre of Islamic Azad University-Kazerun Branch. They housed (5 rats/cage) in a room with controlled light cycle (12L:12D) and temperature (22-24°C) and with access to standard food (regular rat chow, Pars Animal Feed Co., Iran). After 20 days of acclimation, the rats were randomly divided into six groups (214 g mean weight per cage). The rats with approximately same weight were selected to put them in separate groups. During a 20 day acclimation period, the rats in any cages were accessed to 20 g food to determine the amount of food consumption. The rest of the food which remained was weighted every day. The daily food consumption by the rats was 15 g.

### Feeding Regimen

The rats divided into six treatment groups (n = 10). The period of study was 19 days. Control group (Non-FR group) was fed *ad libitum*. Rats in other five groups received 50% of normal food intake (7.5 g). This dosage was determined according to the amount of food consumed in acclimation period and served as Food Restricted (FR) groups. Three of five FR groups were received 100, 200 and 400 mg kg<sup>-1</sup> silymarin (FR-100, FR-200, FR-400 group), respectively. The fourth FR group received placebo (FR-PLA group). The last food restricted

group (FR-CON group) and Non-FR group did not receive any silymarin. A suspension of silymarin was prepared in distilled water and was administered orally for 18 days. Water was available *ad libitum* for all experimental groups.

### Blood Sampling and Measurements

In the last day of the study (19<sup>th</sup> day), all rats were weighted and were anesthetized with ether solution. Heart blood was collected and rats were sacrificed under an overdose ether anesthesia. After clotting at room temperature, serum was separated from blood by centrifuge. Serum samples were labeled for each case and preserved at 20°C for subsequent assays.

Serum glucose, triglyceride, albumin, globulin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, amylase and lipase were measured by commercially available kits (Pars Azemoon, Tehran, Iran) using the Cobas Mira autoanalyzer (Japan). All the biochemical procedures were conducted as described by Burtis and Ashwood (1994).

### Statistical Analysis

Statistical analysis was done using SPSS (2003) software, version 11.5. Results are presented as Mean±SE. Statistical analysis was carried out using one-way ANOVA followed by Tukey test. A probability value less than 0.05 was considered statistically significant.

## RESULTS

### The Effect of Food Restriction and Silymarin on Body Weight of Rats

Food restriction decreased weight of rats ( $p < 0.001$ ) and silymarin treatment did not affect weight of FR rats ( $p > 0.05$ ) (Table 1).

### The Effect of Food Restriction and Silymarin on Serum Parameters

Food restriction and silymarin treatment significantly affected serum triglyceride, AST, ALT, ALP, calcium and amylase levels ( $p < 0.05$ ). Serum concentrations of the other parameters such as glucose, total protein, albumin, globulin, albumin/globulin ratio and lipase were not different among the groups ( $p > 0.05$ ) (Table 2-4).

Table 1: Effects of food restriction and silymarin on body weight of normal weight rats (Mean±SE)

Groups	Body weight*
Non-FR	215.78±8.91 <sup>a</sup>
FR-CON	179.50±4.65 <sup>b</sup>
FR-100	187.56±3.67 <sup>b</sup>
FR-200	188.75±2.57 <sup>b</sup>
FR-400	189.00±1.87 <sup>b</sup>
FR-PLA	181.30±2.14 <sup>b</sup>

\*Dissimilar superscripts in the same column mean significant differences ( $p < 0.001$ ). N = 10 for each group

Table 2: Effects of food restriction and silymarin on serum metabolic factors in the different groups (Mean±SE)

Groups	Glucose	Triglyceride* (mg dL <sup>-1</sup> )	Albumin	Globulin	Albumin/Globulin ratios	Total protein (mg dL <sup>-1</sup> )
Non-FR	112.5±6.47	115.1±9.42 <sup>a</sup>	3.94±0.11	3.96±0.07	0.99±0.04	7.90±0.12
FR-CON	98.1±6.36	60.2±2.28 <sup>b</sup>	3.70±0.08	4.05±0.13	0.91±0.03	7.75±0.15
FR-100	105.9±5.26	86.5±8.20 <sup>c</sup>	3.93±0.07	3.92±0.25	1.06±0.12	7.85±0.25
FR-200	106.9±6.61	74.9±4.76 <sup>bc</sup>	3.81±0.06	4.30±0.17	0.89±0.04	8.11±0.17
FR-400	93.0±7.18	62.4±2.98 <sup>bc</sup>	3.73±0.07	4.06±0.10	0.92±0.04	7.79±0.10
FR-PLA	92.4±5.47	58.5±1.51 <sup>bc</sup>	3.75±0.05	4.32±0.10	0.87±0.02	8.07±0.07

\*Dissimilar superscripts in the same column mean significant differences ( $p < 0.001$ )

Table 3: Effects of food restriction and silymarin on serum hepatic enzymes in the different groups (Mean±SE)

Groups	AST* (IU L <sup>-1</sup> )	ALT* (IU L <sup>-1</sup> )	ALP* (IU L <sup>-1</sup> )
Non-FR	206.70±27.36 <sup>a</sup>	85.90±7.60 <sup>a</sup>	326.60±44.15 <sup>a</sup>
FR-CON	188.70±17.43 <sup>b</sup>	53.80±3.98 <sup>b</sup>	169.60±19.89 <sup>b</sup>
FR-100	278.00±24.55 <sup>b</sup>	69.50±4.16 <sup>ab</sup>	182.80±13.24 <sup>b</sup>
FR-200	204.70±18.27 <sup>ab</sup>	63.90±4.46 <sup>b</sup>	215.60±15.83 <sup>b</sup>
FR-400	248.60±15.98 <sup>ab</sup>	67.60±3.52 <sup>ab</sup>	223.30±20.90 <sup>b</sup>
FR-PLA	172.40±12.83 <sup>ab</sup>	58.50±5.74 <sup>b</sup>	195.90±18.42 <sup>b</sup>

\*Dissimilar superscripts in the same column mean significant differences (p≤0.005)

Table 4: Effects of food restriction and silymarin on serum pancreatic factors in different groups (Mean±SE)

Groups	Amylase* (IU L <sup>-1</sup> ) (p = 0.001)	Lipase (IU L <sup>-1</sup> )	Calcium** (mg dL <sup>-1</sup> ) (p<0.05)
Non-FR	2317.5±234.07 <sup>a</sup>	4.1±1.45	9.93±0.42
FR-CON	1463.3±81.68 <sup>b</sup>	1.9±0.35	9.33±0.52
FR-100	1837.0±120.77 <sup>ab</sup>	2.0±0.42	8.35±0.20
FR-200	1725.4±84.16 <sup>b</sup>	1.7±0.21	8.84±0.40
FR-400	1624.2±89.63 <sup>b</sup>	2.0±0.33	8.39±0.55
FR-PLA	1610.4±102.49 <sup>b</sup>	4.1±1.54	9.62±0.31

\*, \*\*Dissimilar superscripts in the same column mean significant differences

Results showed that food restriction decreased serum triglycerides in all FR groups (p<0.01). Administration of 100 mg kg<sup>-1</sup> of silymarin increased serum triglycerides levels compared to FR-400, FR-CON and FR-PLA groups (p<0.05), although, these levels were not different between FR-100 and FR-200 groups (p>0.05) (Table 2).

Food restriction did not significantly change AST levels compared to Non-FR group. Silymarin treatment increased AST levels in FR-100 group compared to FR-CON and FR-PLA groups (p≤0.05) (Table 3).

Results represented that food restriction decreased ALT and ALP levels. Nevertheless, there was no significant difference between these enzyme levels in silymarin-treated and untreated FR groups (p>0.05) (Table 3).

The pancreatic enzymes (amylase and lipase) and calcium results were showed in Table 4. The compare of serum amylase levels in FR and non-FR groups shows the significant decrease of serum amylase, except in FR-100 group (p<0.05). Administration of silymarin did not significantly change amylase levels.

## DISCUSSION

Results showed that food restriction decreased body weight of the rats although silymarin did not affect it. It is possible that the longer period of the study can cause to specify the effect of silymarin on the body weight.

Present results represented that food restriction and silymarin could not change serum glucose levels. Similar result was found by other researchers who worked on food restriction conditions (Taylor *et al.*, 1974; Cleary *et al.*, 1987; Noblot *et al.*, 2001). Also, silymarin could not change serum glucose levels in FR rats. The previous reports in experimental or natural diabetes mellitus in human and rats showed that silymarin decreases blood glucose (Soto *et al.*, 2004; Huseini *et al.*, 2006; Vengerovskii *et al.*, 2007). It has been suggested that silymarin decreased serum glucose by influencing on pancreatic and hepatic functions (Soto *et al.*, 2004; Kren and Walterov, 2005).

In our previous study on pregnant FR rats, food restriction increased serum glucose levels and administration of silymarin to FR rats cause to decrease serum glucose levels (Mahjoor and Dehghan, 2008). These results suggest that silymarin is not a hypoglycemic agent in all condition and has a modulatory action.

Results indicated that food restriction could significantly decrease serum triglyceride concentrations. Similar result was found by Fan *et al.* (2003) in FR rats which were on high fat diet previously.

Silymarin is known as a hypocholesteremic drug which can decrease blood triglyceride concentration (Skottova and Krecman, 1998). We hypothesized that silymarin can induce further reduction in serum triglyceride concentrations in food restriction condition, but our results revealed that silymarin (100 mg) could increase its concentrations in FR rats.

These discrepancies among our results and previous studies about glucose and triglyceride have a relation to this fact that maybe silymarin is a modulator of metabolic parameters such as glucose and triglyceride, although it has been suggested that silymarin is a hypocholesterolemic and hypoglycemic agent (Soto *et al.*, 2003, 2004; Skottova and Krecman, 1998; Huseini *et al.*, 2006; Vengerovskii *et al.*, 2007; Skottova *et al.*, 1998, 2003).

Hepatic enzymes increase in hepatic injuries (Stockham and Scott, 2002). It has been observed the increase of blood level of hepatic enzymes in caloric restriction in rats with obesity and hyperlipidemia (Fan *et al.*, 2003). Results of the present study demonstrated that food restriction in normal weight rats did not increase hepatic enzymes, but serum ALT and ALP levels decreased in the same condition. The levels of these enzymes did not change by silymarin in FR rats. The decrease of serum ALT and ALP levels in FR groups may relate to decrease in liver functional mass in FR rats (Stockham and Scott, 2002; Dumas *et al.*, 2004), although, we did not weight the livers and further studies are needed.

Serum amylase levels in FR groups were significantly lower than those which were in Non-FR group. Also, lipase and calcium levels in serum were not differed between FR and Non-FR groups. The increasing of serum amylase and lipase and hypocalcemia were found in pancreas abnormalities (Rattner *et al.*, 1990). Our results did not show any pancreas abnormality.

Protective activities of silymarin on liver and pancreas have well been known in pathologic and toxicological conditions (Soto *et al.*, 2004; Kren and Walterov, 2005; Ramsey *et al.*, 2000). Also, moderate food restriction has been recommended to treat some liver and pancreas problems (Fan *et al.*, 2003; Noblot *et al.*, 2001). It seems that silymarin and food restriction concurrently have more beneficial effects on liver and pancreas problems, which need more exact studies.

In conclusion, the results showed no constant decrease on serum glucose and triglyceride by silymarin. Also, concurrent use of silymarin and food restriction can modulate obvious changes in metabolic factors, which needs further studies.

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