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Effect of Silymarin on Metabolic Factors of Food-Restricted Over Conditioned Wistar Rats

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Abstract: This study was conducted to evaluate the changes in serum metabolic factors of over conditioned pregnant rats treated with silymarin in food restriction condition. Sixty pregnant Wistar rats were divided into five equal groups. All rats received high energy diet before treatments. 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 food-restricted (FR) groups. Three of five FR groups received 150, 200 and 400 mg kg^{-1} silymarin, respectively (FR-150, 200, 400). Another FR group (FR-Con) and the Non-FR group did not receive any silymarin. Glucose, triglyceride, LDL and HDL cholesterol, total cholesterol, thyroid hormones and cortisol were measured in serum. All factors were significantly different between groups except free-T_4 and T_4. Serum glucose concentrations in FR-150 and 200 and Non-FR groups were lower than FR-Con and FR-400. Silymarin significantly increased serum triglycerides, LDL cholesterol and cholesterol contents in FR groups. The highest levels of these factors were noted in 200 mg silymarin-treated group. HDL cholesterol was highest in FR-Con; meanwhile FR-200 group had the lowest HDL cholesterol. Serum cortisol decreased in treated and untreated FR groups except FR-150 group. Free-T_3 and T_3 concentrations in FR-400 and FR-Con groups were higher than the other silymarin treated groups. In conclusion our results indicate that 200 mg kg^{-1} of silymarin in Wistar rats is the best dosage to achieve metabolic benefits. Silymarin has positive effects on lipid metabolism and can modulate serum triglyceride and cholesterol concentrations in food restriction condition. Also, the present findings suggest that silymarin under food restriction situation exerts a decreasing effect upon peripheral conversion of T_4 to T_3.

Key words: Silymarin, food restriction, over condition, food restriction, rat

INTRODUCTION

Milk thistle (*Silybum marianum* L.) is native to the Mediterranean basin and is now widespread throughout the world. Silymarin, a natural hepatoprotector, is a standardized extract of *Silybum marianum* that is composed of a mixture of flavonolignanes in which silibinin is the main compound (Gopal and Sengottuvelu 2008). Silymarin has a range of metabolic properties (Fraschini *et al*., 2002; Saller *et al*., 2007). Silymarin is a strong antioxidant that has been proved to promote liver cell regeneration, reduce blood cholesterol and to help prevent cancer (Soto *et al*., 2004).

The aim of the study was to determine the effects of silymarin on metabolic factors and thyroid hormones in over conditioned rats were in food-restricted situations.

MATERIALS AND METHODS

The study was conducted in 2007, May and June, in animal house of research center of Islamic Azad University-Kazeroon Branch, Iran.

Silymarin extract (82% purity and minimum 30% silibinin) was bought 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).

Sixty female Wistar rats were prepared from animal house of research center of Islamic Azad University-Kazeroon Branch. They housed (3 rats/cage) in a room with controlled light cycle (12L/12D) and temperature

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(22-24°C). All rats received high energetic diet during 20 days of acclimation period. The females were housed with mature, fertile male rats until pregnancy was confirmed by vaginal smear. A positive score denoted to the presence of a copulatory plug in the vagina and/or spermatozoa in vaginal smears. Once pregnancy was confirmed, the rats were placed in standard plastic laboratory maternal tubs and assigned to one of five gestational treatment groups.

Feeding regimen: The pregnant rats were divided randomly into five groups (~215 g mean weight per cage and 3 rats per cage) (no in each group = 12). The rats accessed to standard food (regular rat chow, Pars Animal Feed Co., Iran) during treatment period.

The period of the study on pregnant rats was 19 days. Control group (Non-FR group) was fed ad libitum. Rats in four other groups received 50% of normal food intake, as determined by the amount of food consumed in preliminary study and served as food-restricted (FR) groups. During the gestation period, food consumption of Non-FR rats was determined and the FR rats in any cages were accessed to 50% of Non-FR diet (Leonhardt *et al*., 2003).

Three of five FR groups received 150, 200 and 400 mg kg^{-1} silymarin (FR-150, FR-200, FR-400 group), respectively. 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 always available ad libitum for all experimental groups.

Blood sampling and measurements: In the last day of the study (19th day), all rats were weighted and thereafter were anesthetized with ether solution. Heart blood of the rats was collected and euthanasia was performed later. Serum was separated after clotting at room temperature by centrifuge. Serum samples were labeled for each case and preserved at -20°C for subsequent assays.

Serum glucose, triglyceride, LDL and HDL cholesterol and total cholesterol were measured using commercial kits (Pars Azemoon, Tehran, Iran) by calorimetric methods.

Serum concentrations of cortisol, total T_3 and T_4 were determined by radioimmunoassay method (RIA kit, Immunotech, Czech Republic). Serum concentrations of free T_3 and free T_4 were determined by competitive ELISA assay (AccuBind ELISA kit, Monobind Inc., USA).

Statistical analysis: Statistical analyses were done using SPSS software, version 11.5. Results are presented as mean±SE. Statistical analysis were carried out using One-Way ANOVA followed by Tukey test. The $p<0.05$ was considered as statistically significant.

RESULTS

The effect of food restriction and silymarin on weight and litter size: Food restriction decreased weight of pregnant rats at the end of the study ($p = 0.002$). Silymarin treatment did not affect weight of FR rats ($p>0.9$) (Table 1).

Non-Food restricted rats had marginally lower litter size than FR rats, although, weight of fetuses were not significantly different between groups (Table 2).

The effect of food restriction and silymarin on metabolic factors: Food restriction and silymarin treatment significantly affected serum glucose, triglyceride, LDL and HDL cholesterol and cholesterol levels ($p<0.001$) (Table 3).

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

	Body weight	
Groups	Before treatment	After treatment*
Non-FR	210.17±3.81	277.83±11.48^{a,b,c}
FR-CON	217.83±4.88	232.50±9.45^{a}
FR-150	214.92±3.91	232.83±8.40^{b}
FR-200	216.17±3.18	243.83±6.65
FR-400	216.58±2.68	226.67±9.06^{c}

*Different significantly between groups ($p<0.01$), Means in the same column with same lower case superscripts are significantly different

Table 2: Effects of food restriction and silymarin on number and weight of fetus in over conditioned rats (Mean±SE)

Groups	Non-FR	FR-CON	FR-150	FR-200	FR-400
Fetus number*	8.25±1.00	11.2±0.92	9.75±1.00	11.82±0.87a	11.75±0.59b
Fetus weight	58.72±3.69	53.1±5.29	50.69±3.59	50.57±5.38	51.03±5.24

*Different significantly between groups ($p = 0.04$), a: marginally different with Non-FR group ($p = 0.05$ and $p = 0.089$, respectively)

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

Groups	Glucose* (mg dL^{-1})	Triglyceride* (mg dL^{-1})	LDL cholesterol* (mg dL^{-1})	HDL cholesterol* (mg dL^{-1})	HDL/ LDL cholesterol ratio	Cholesterol* (mg dL^{-1})
Non-FR (n = 12)	155.25±5.15^{a,d}	88.83±3.28^{a,b}	9.25±0.53^{a}	34.75±2.06^{a}	3.98±0.42	63.67±3.5^{a}
FR-CON (n = 10)	198.60±6.97^{a,b,c}	76.10±4.20^{c,d}	9.19±0.75^{b}	46.00±3.60^{a,b}	5.27±0.50	68.40±3.62^{b}
FR-150 (n = 10)	135.10±6.17^{b}	103.56±5.84	9.01±0.65	38.50±3.27	4.53±0.87	71.60±3.34
FR-200 (n = 11)	147.18±5.73^{c}	279.82±15.45^{a,c}	19.74±1.60^{a,b}	27.90±1.94^{b}	1.50±0.15	88.97±5.18^{a,b}
FR-400 (n = 11)	194.60±4.43^{d}	179.73±10.42^{b,d}	12.65±1.58	37.09±3.01	6.88±4.15	69.45±3.64

*Different significantly between groups ($p<0.001$), Means in the same column with same lower case superscripts are significantly different. The differences were shown only between silymarin-treated groups and Non-FR and FR-CON groups

Table 4: Effects of food restriction and silymarin on serum metabolic hormones in the different groups (Mean±SE)

Groups	Cortisol* (ng mL^{-1})	Free T_3* (ng L^{-1})	Free T_4 (ng L^{-1})	T_3* (nmol L^{-1})	T_4** (nmol L^{-1})
Non-FR (n = 12)	43.33±1.78^{a,b,c}	9.91±0.72^{a}	25.67±1.36	1.73±0.10	81.67±4.85
FR-CON (n = 10)	21.56±1.94^{a,d}	10.87±0.72^{b}	30.71±1.47	1.97±0.08^{a,b}	90.50±5.60
FR-150 (n = 10)	44.29±2.97^{d}	9.62±0.70	24.98±2.78	1.45±0.15^{a}	80.50±7.39
FR-200 (n = 11)	28.18±1.92^{b}	7.53±0.63^{b}	26.97±1.35	1.35±0.11^{b}	72.55±5.82
FR-400 (n = 11)	29.15±2.33^{c}	13.31±0.59^{a}	28.12±0.94	1.95±0.11	94.18±5.01

*Different significantly between groups ($p<0.001$), **Different marginally between groups ($p = 0.076$), Means in the same column with same lower case superscripts are significantly different. The differences were shown only between silymarin-treated groups and Non-FR and FR-CON groups

Serum glucose levels in FR-CON and FR-400 groups were higher than Non-FR, FR-150 and FR-200 groups ($p<0.001$). Results showed that serum triglycerides in Non-FR, FR-CON and FR-150 groups were significantly lower than FR-200 and FR-400 groups. Also, the FR-200 group had the highest levels in all groups.

Serum LDL cholesterol and cholesterol levels in FR-200 group were significantly higher than all the other groups. Serum HDL cholesterol levels in FR-CON group were significantly higher than Non-FR and FR-200 groups. The HDL/LDL cholesterol ratio was not significantly different between groups.

The effect of food restriction and silymarin on metabolic hormones: Food restriction and silymarin treatment significantly affected serum cortisol and free T_3 and T_3 levels ($p<0.001$). Although, T_4 levels were marginally different between groups ($p = 0.076$), the free T_4 levels were not different between groups ($p>0.05$) (Table 4).

Serum cortisol levels in Non-FR and FR-150 groups were significantly higher than FR-CON, FR-200 and FR-400 groups.

Serum levels of free T_3 in FR-400 were higher than Non-FR, FR-150 and FR-200 ($p<0.01$). Also, these levels in FR-CON group were significantly higher than FR-200 group. Serum concentrations of T_3 in FR-CON and FR-400 groups were significantly higher than FR-150 and FR-200 groups.

DISCUSSION

In comparison of FR and Non-FR pregnant rats, our results represented that food restriction, in term pregnant rats, increased serum glucose and HDL cholesterol levels, whereas, there was no difference between levels of triglyceride, LDL cholesterol and cholesterol. Also, these results showed that administration of silymarin to FR rats decreased serum glucose and HDL cholesterol levels, while, triglyceride, LDL cholesterol and cholesterol levels increased.

Spindler *et al.* (2003) noted that caloric restriction reduces blood glucose and insulin concentrations in rodents and suggested that expression of some gluconeogenesis enzymes increases in caloric restriction. Our results represent that serum glucose levels increase in FR rats compare to Non-FR rats. Higher level of glucose in FR-CON group may relate to increase in glucocorticoids and metabolic stress during the end of pregnancy (Nyirenda *et al.*, 2001).

The present study results shows that silymarin (150 and 200 mg/kg/day) may inhibit increase of serum glucose levels in food restriction condition. It was reported that silymarin reduces blood glucose in experimental or natural diabetes mellitus in human and rats (Soto *et al.*, 2004; Vengerovskii *et al.*, 2007). This effect can be due to this fact that silymarin recovers pancreatic function (Soto *et al.*, 2004). Also, it was found that silymarin was able to prevent rise in plasma glucose by its effect on the liver (Kren and Walterov, 2005).

It has been suggested that silymarin and silibinin could significantly decrease serum triglyceride concentrations and posse a hypocholesterolemic effect in rats fed high cholesterol diet (Skottova and Krecman, 1998). It was found that serum triglyceride concentrations decrease in FR rats which have received high fat diet (Fan *et al.*, 2003). These findings suppose that concurrent use of silymarin and food restriction enhance decreasing of serum triglyceride concentrations. But our results revealed that food restriction in rats, at the end of pregnancy; do not significantly decrease triglyceride concentrations. Silymarin (200 and 400 mg) increased triglyceride concentrations in these FR rats. Multiplying effect of silymarin on serum triglyceride concentration was observed in our previous studies in food- restricted non-pregnant rats (Unpublished data).

Our results showed that administration of 200 mg silymarin in FR rats significantly increases serum triglyceride, LDL cholesterol and cholesterol and these levels reached higher than Non-FR group, although, this dosage reduced HDL cholesterol in FR rats.

In other studies Skottova *et al.* (2003) and Sobolova *et al.* (2006) the influence of silymarin and its polyphenolic fraction in rats fed on high cholesterol diet were evaluated. Silymarin significantly reduced cholesterol absorption in these rats. Sobolova *et al.* (2006) found that administration of silymarin in high cholesterol

diet significantly reduced total cholesterol in plasma and caused more than twofold decrease of VLDL cholesterol compared to high cholesterol diet, although, Skottova *et al.* (2003) demonstrated that silymarin did not alter the total cholesterol content in plasma. Sobolova *et al.* (2006) unlike to Skottova *et al.* (2003) found that feeding on high cholesterol diet induced hypercholesterolemia.

It was found that the level of HDL cholesterol was significantly higher in rats fed silymarin (Sobolova *et al.*, 2006), although, Skottova *et al.* (2003) found that silymarin showed only negligible HDL cholesterol increasing effects.

It was observed that silymarin did not have any influence on LDL cholesterol levels (Skottova *et al.*, 2003; Sobolova *et al.*, 2006). These results suggested that the inhibition of cholesterol absorption caused by silymarin and its polyphenolic fraction could be a mechanism contributing to the positive changes in plasma cholesterol lipoprotein profile and in lipid content in liver (Sobolova *et al.*, 2006).

Skottova *et al.* (2003) observed that the ratios of HDL cholesterol to VLDL cholesterol (largely dose-dependently) increased, although, in our study there was not any significant difference between HDL to LDL cholesterol in different groups.

It was suggested that effects of silymarin were dose-dependent and positively modified lipoprotein profile counteract the development of fatty liver (Skottova *et al.*, 2003).

This controversy among our results and the others (Skottova and Krecman, 1998; Skottova *et al.*, 2003; Sobolova *et al.*, 2006) may relate to difference between the study conditions. Pregnancy develops a lot of hormonal and metabolic changes in pregnant animals. Food restriction during pregnancy has different effects in different periods, especially at term. Therefore, metabolic effects of silymarin in pregnant animals should be studied more. Nevertheless, it seems that silymarin does not have an absolute decreasing effect on blood triglyceride and cholesterol concentrations (Skottova and Krecman, 1998) and silymarin plays a role as a modulator of blood triglyceride in different situations. Skottova *et al.* (2003) found that the rats fed standard laboratory diet did not respond to administration of silymarin and only a mild increase in HDL cholesterol was found in these rats.

In comparison of FR and Non-FR pregnant rats, our results showed that food restriction increased serum cortisol levels, while, the levels of thyroid hormones were not different. Previous results showed that dietary restriction decreased levels of T_3, Free T_3 and Free T_4 in pregnant rats (Hastings and Zeman, 1979; Oberkotter and Rasmussen, 1992). Although, Oberkotter and Rasmussen (1992) found that food restriction decreased T_4 levels in pregnant rats, Hastings and Zeman (1979) did not find any changes.

This discrepancy between our results and previous studies may relate to the diet used (Hastings and Zeman, 1979) or time of blood sampling (Oberkotter and Rasmussen, 1992).

The present study demonstrated that administration of silymarin to FR rats decreased serum T_3 and Free T_3 levels, whereas, the levels of cortisol, T_4 and Free T_4 did not changed. Similar study was not found any where. These results may relate to effect of silymarin on peripheral conversion of T_4 to T_3.

The results showed that dosage of 200 mg kg^{-1} silymarin had significant effects on almost all of metabolic factors which measured, while, dosage of 400 mg kg^{-1} had lowest significant effects on these factors. These results demonstrated that the serum levels of metabolic factors in FR-400 group were similar to FR-CON group which may relate to laxative effect of silymarin in higher dosage (Wellington and Jarvis, 2001).

In conclusion, our results indicate that silymarin has positive effects on lipid metabolism and can modulate serum triglyceride and cholesterol concentrations in food restriction condition. Also, the present findings suggest that silymarin under food restriction situation exerts a decreasing effect upon peripheral conversion of T_4 to T_3.

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