Original Article

Use of Silymarine as Adjuvant in Type 1 Diabetes Mellitus Patients Poorly Controlled with Insulin

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Summary:

Background: Glycemic control and prevention of secondary complications are the most important aims of using pharmacological treatments in diabetes mellitus. Due to the high incidence of inadequate response to insulin and, we try to evaluate the effects of adjunct use of silymarin with insulin on glycemic control, lipid profile and renal function in type 1 diabetes mellitus patients.

Patients and Methods: Placebo-controlled, doubled blinded clinical trial method is utilized through which 60 type 1 DM patients allocated into two groups, 30 patients treated with insulin and silymarin 400 mg/day in two divided doses, while the other 30 patients treated with insulin and placebo for 60 days. Fasting serum glucose, HbA1c, C-peptide, lipid profile and microalbuminuria were evaluated at base line and after 60 days.

Results: Compared with placebo, silymarin significantly improves the effects of insulin through the reduction of fasting serum glucose, HbA1c and increase C-peptide level, associated with improving lipid profile and renal function.

Conclusion: Adjunct use of silymarin with insulin improves glycemic control associated with improving lipid profile and renal function, an effect that may be related to increased insulin sensitivity in peripheral tissues.

Keywords: Silymarin, Type1 DM, Glycemic control

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Introduction:

Diabetes mellitus is the most common serious metabolic disorder, and considered to be one of the five leading causes of death in the world (1). It can be managed by diet, exercise, and drug therapy. However, the pharmacologically active agents are either too expensive or have undesirable side effects or contraindications (2). Throughout the world, many traditional plant treatments for diabetes exist, and therein lies a hidden wealth of potentially useful natural products for the control of diabetes (3). Oxidative stress is known to be a common factor in the pathogenesis of diabetes and its complications (4). There is evidence for multiple pathways of increased generation of reactive oxygen species (ROS) in diabetes. During hyperglycemia, an increased glucose load elevates non-enzymatic glycosylation, irreversible cross-linking of certain proteins, lipid peroxidation, and loss of function in human red blood cells (5, 6). Markers of oxidative stress and reduced levels of Antioxidants have been found in blood and/or tissues, including kidney, in both human and experimental animal diabetes (7, 8). *Silybum marianum* (L.) is a member of the *Asteraceae* family and its seed extract contain large numbers of chemical constituents including several flavonoignans collectively known as silymarin (9). Silymarin has powerful antioxidant properties and has beneficial effect on various hepatic disorders, including hepatotoxicity secondary to acute and chronic viral hepatitis, mushroom poisoning, and alcoholic cirrhosis (10, 11). The present study was designed to investigate the efficacy silymarin on glycemic control in type1 diabetic patients poorly controlled with exogenously administered insulin.

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Patients and methods:

This study was performed on 60 patients (39 males and 21 females) with type 1 diabetes mellitus (DM); their age range was 25-73 (44.32 ± 16.49) years with disease duration of more than 3 years. They are treated with 30-50 units/day of insulin but with mild response and poor glycemic control. The patients were maintained on dietary control program under the supervision of clinical nutrition specialist at the Specialized Center for Diabetes and Endocrinology in Baghdad, during the period from February 2007 to

January 2008, and the study protocol was approved by its scientific committee. All the selected patients had no other marked pathologic disorders such as hypertension and ischemic heart diseases as revealed by the clinical investigation. All patients were selected according to the following criteria: they did not have other associated chronic diseases such as liver or kidney disorders and no cardiovascular complications. Patients who were pregnant or breast-feeding were excluded. The patients were enrolled in a double-blind, placebo controlled study, and randomized 1:1 pattern into silymarin treated and placebo treated groups. The patients were allocated into 2 groups as follow: Group A, includes 30 patients with type 1 diabetes mellitus treated with 400mg/day Silymarin given in divided doses (each 12 hour) for 60 days in a capsule dosage form specially prepared for this purpose, in addition to their insulin therapy; group B, includes 30 patients with type 1 diabetes mellitus treated with a placebo formula each 12 hour for 60 days in a capsule dosage form, in addition to their insulin therapy. After 12 hours fasting, blood samples were collected from all patients by vein puncture, before starting drug treatment (as baseline samples) and then after 60 days to follow the changes in the studied parameters. Blood samples were divided immediately into different types of tubes, either in EDTA-containing tubes for the determination of glycated hemoglobin (12), or in plane tubes for preparation of serum to evaluate plasma glucose level (13), C-peptide level (14), triglyceride levels (15), total cholesterol (16), HDL-c and LDL-c level (17). Additionally, urine samples were collected from all patients for determination of microalbuminuria (18). Student's paired *t*-test and ANOVA test were used to examine the degree of significance, and *P* values less than 0.05 were considered significantly different.

Results:

The data presented in table 1 showed that treatment with silymarin (group A) in addition to insulin therapy resulted in a significant reduction ($P<0.05$) in FPG levels (26.1%) after 60 days compared to base line levels. Meanwhile, in the group received placebo formula, non-significant ($P>0.05$) increase in FPG levels (4.3%) was reported compared to base line value after 60 days of treatment. The effects of the two different approaches of treatment on the FPG levels after 60 days showed that adjuvant use of silymarin produced significantly higher reduction in FPG levels compared to placebo. Table 1 also indicated that adjuvant treatment with silymarin (group A) resulted in a significant reduction ($P<0.05$) in glycated hemoglobin (19.9%) after 60 days compared to base line levels. While in group B, no placebo effects were observed. When the effects of the two approaches of treatment were compared, adjuvant use of silymarin produced significantly higher reduction in HbA_{1c} level compared to using placebo with insulin. Concerning the effect on endogenous insulin secretion, measured as C-peptide levels, silymarin produced significant elevation in C-peptide levels (47.2%, $P<0.05$) compared to baseline values after 60 days. Meanwhile, addition of placebo to the therapeutic regimen did not significantly affect endogenous insulin secretion (16.4%, $P>0.05$) compared to baseline level. When both types of treatment compared, silymarin improves secretion of endogenous insulin compared to placebo formula. Table 2 showed that use of silymarin with insulin resulted in significant reduction ($P<0.05$) in total cholesterol (TC) levels (13.0%) after 60 days. Meanwhile, in placebo treated diabetic patients, insulin treatment did not reduce total cholesterol levels, but significant elevation was observed (28.1%, $P< 0.05$) compared to baseline levels. Comparison of percent changes in total serum cholesterol levels due to the two modes of treatment followed revealed significant difference between both groups. Table 2 also showed that both silymarin and placebo have non-significant effect ($P>0.05$) on serum triglycerides (TG) levels after 60 days compared to baseline levels. Table 2 revealed also that serum LDL levels in type 1 DM patients were significantly reduced ($P<0.05$) as a result of adjuvant use of silymarin (19.3%) after 60 days compared to baseline values; while group B diabetic patients showed no significant changes compared to baseline values. Comparison of percent changes in serum LDL levels due to the effects of the two modes of treatment showed that silymarin resulted in significant effect in reducing serum LDL levels compared to placebo. Finally, table 2 showed that use of silymarin with insulin significantly elevates HDL-c levels (38%, $P<0.05$) compared to baseline value. Moreover, group B diabetic patients showed no significant changes in serum HDL-c levels during the treatment period compared to baseline value. When the percent changes in HDL-c levels in both groups were compared, addition of silymarin 400 mg/day improves HDL-c levels significantly compared to the use of insulin alone. Microalbuminuria was significantly reduced ($P<0.05$) due to treatment with silymarin as an adjuvant therapy with insulin (67.4%) after 60 days compared to base line levels (table 3). In placebo-treated patients, microalbuminuria levels were significantly elevated during treatment (8.9%) compared to baseline values. Comparison between the effects of the two approaches of treatment on the microalbuminuria clearly showed that silymarin significantly reduced the levels of microalbuminurea compared to placebo.

Table (1): Effects of treatment with 400 mg/day Silymarin and Insulin on Fasting plasma glucose (FPG), Glycated hemoglobin (HbA1c) and C-peptide levels in type 1 DM patients:

Patient group	Fasting plasma glucose (mm mol/L)		(HbA1c) %		C-peptide ng/mL	
	Baseline	After 60 days	Baseline	After 60 days	Baseline	After 60 days
Group A Silymarin + Insulin n = 30	16.3 ± 1.2	12.0 ± 1.0*[a]	13.3 ± 0.1	10.6 ± 0.7*[a]	3.2 ± 0.38	3.3 ± 0.34 [a]
Group B Placebo + Insulin n = 30	15.1 ± 2.0	15.8 ± 1.7 [b]	12.6 ±1.2	12.6 ± 1.0 [b]	2.4 ± 0.14	2.0 ± 0.20 [b]

Values are presented as mean ± SEM; n = number of patients; * Significantly different compared to base line (P<0.05); Values with non-identical superscripts (a,b) among different groups are considered significantly different (P< 0.05).

Table (2): Effects of treatment with 400 mg/day Silymarin and Insulin on the Serum Lipid profile (Cholesterol, Triglyceride, LDL-c and HDL-c) levels in type 1 DM patients:

Patient group	Serum cholesterol mmol/L		Serum triglyceride mmol/L		Serum LDL-c mmol/L		Serum HDL-c mmol/L	
	Baseline	After 60 days	Baseline	After 60 days	Baseline	After 60 days	Baseline	After 60 days
Group A Silymarin + Insulin n = 30	4.7 ± 0.23	4.1 ± 0.29 *	1.7 ± 0.14	2.0 ± 0.23	2.2 ± 0.14	1.8 ± 0.19*[a]	2.1 ± 0.25	1.8 ± 0.18 [a]
Group B Placebo + Insulin n = 30	3.5 ± 0.23	4.4 ± 0.21*	1.8 ± 0.17	1.9 ± 0.22	2.0 ± 0.26	2.4 ± 0.08 [b]	2.3 ± 0.41	2.2 ± 0.44 [b]

Values are presented as mean ± SEM; n = number of patients; * significantly different compared to base line (P < 0.05); Values with non-identical superscripts (a,b) among different groups are considered significantly different (P < 0.05).

Table (3): Effects of treatment with 400 mg/day Silymarin and Insulin on Microalbuminuria (MUA) levels in type 1 DM patients:

Patient group	Microalbuminuria (mg /L)	
	Base line	After 60 days
Group A Silymarin + Insulin n = 30	67.53 ± 5.25	22.02 ± 2.08*[a]
Group Placebo + Insulin n = 30	46.09 ± 2.67	50.22 ± 5.96*[b]

Values are presented as mean ± SEM; n = number of patients; *Significantly different compared to base line (P<0.05); Values with non-identical superscripts (a,b) among different groups are considered significantly different (P<0.05)

Discussion:

Although insulin treatment regimen is followed in case of type 1 DM patients, glycemic control is not achieved, revealed as excessively elevated FPG and HbA_{1c} levels, this might be attributed to disturbances in the response of target tissues to the effect of exogenously administered insulin doses and/or immunologic response initiated by the biological system against insulin; adjuvant use of silymarin with insulin improves the response to insulin, revealed as significant decrease in both FPG and HbA_{1c} levels compared to placebo (19). In a clinical trial conducted by Velussi *et al*, silymarin decreases the requirement for administration of exogenous insulin in DM patients with liver cirrhosis compared to placebo (20). Within the limitations of our literature review, no reported

data on the effect of silymarin in type 1 DM observed in clinical setting; all available data are concerned with the effect *in vitro* or in experimental animal models of diabetes. Silymarin prevents the rise in both plasma glucose and pancreatic lipid peroxidation in hyperglycemic rats (21). Thus, it was suggested that the protective effect could be attributed either to its antioxidant properties or to an increase of plasma and pancreatic glutathione concentrations, or both. Silymarin also stimulated pancreatic activity of many antioxidant enzymes like glutathione peroxidase, superoxide dismutase and catalase (22). Silymarin has not only a protective effect against alloxan-induced diabetes mellitus in rats but also induced pancreatic recovery (22), and this effect may explain the reported increase in insulin secretion from the pancreas in type 1 DM patients (table 1) due to adjuvant use of silymarin with insulin. Accordingly, silymarin may represent a new approach in the treatment of diabetes mellitus, not only for the enhancement of insulin activity but also for the recovery of pancreatic function. Therefore, it may be useful as a therapeutic agent for type 1 DM. In the present study, adjuvant use of silymarin significantly improves the impaired lipid profile, revealed by decreasing total cholesterol and LDL-c levels, while triglyceride and HDL-c levels were not significantly changed. Administration of silymarin to rats with impaired lipid profile results in significant reduction in total cholesterol and LDL-c levels associated with significant elevation in HDL-c levels (23), a profile of effects consistent with that reported in the present study. Moreover, Kercman *et al* reported that silymarin inhibits development of hypercholesterolemia in rats fed cholesterol-rich diet and compared with that produced by probucol, associated with increase in HDL-c levels and decrease in the liver content of cholesterol (24). Accordingly, the mechanisms through which silymarin elicited its regulating effect may be attributed to its pleotropic effects that include improving glycemic control, limiting absorption of bile acids (25), inhibition of the enzyme HMG-CoA reductase and powerful antioxidant properties (26). It has been reported that in patients with both types of diabetes mellitus, especially those with poor glycemic control, microalbuminuria predicts both progression to end stage renal disease and an increase in cardiovascular mortality compared to diabetic patients without microalbuminuria (27). This difference in risk can be related to the presence of glycated proteins which are directly toxic to both renal and vascular tissues through stimulation of reactive oxygen species (ROS); in this respect, treatment with antioxidants (such as flavonoids or others) significantly reduces the development of diabetic nephropathy (28). Microalbuminuria levels (indicator of microvascular changes) were elevated in type 1 DM patients (tables 3), results which are compatible with those reported by Lepore *et al*, where patients with HbA_{1c} levels <7% presented lower prevalence of macro and microvascular complications than patients with HbA_{1c} >9% that mostly associated with microalbuminuria and diabetic nephropathy (29). Adjuvant use of silymarin with insulin significantly reduces microalbuminuria levels in type 1 DM patients. Many previously reported data indicated the effects of silymarin and its major component silibinin against renal tissue toxicity induced *in vitro* or *in vivo* by many drugs including gentamicin (30). This nephroprotective effect may be attributed to the powerful antioxidant and cytoprotective properties of silymarin. In conclusion, adjuvant use of silymarin during treatment of type 1 diabetes mellitus with insulin improves glycemic control, lipid profile and renal function compared to placebo.

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