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RESEARCH ARTICLE

Effect of Silymarin Supplementation on Glycemic Control, Lipid Profile and Insulin Resistance in Patients with Type 2 Diabetes Mellitus.

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Abstract

Background: Suboptimal glycemic control and insulin resistance are common situations in diabetes. This is associated with high risk of macrovascular and microvascular complications. Silymarin, an oral hepatoprotective herbal medicine with antioxidant and anti-inflammatory properties, is suggested as an attractive candidate for the treatment of diabetes and its complications.

Aim: To evaluate the efficacy of silymarin as adjuvant therapy to standard anti-diabetic treatment on glycemic control, lipid profile and insulin resistance compared to standard treatment alone in patients with type 2 diabetes mellitus.

Patients and Methods: A Prospective, Randomized, Placebo- Controlled, Single -Blinded, Pilot study was conducted. Forty patients were randomly assigned to receive either silymarin capsules 140 mg three times daily (n=20) or identical placebo capsules three times daily (n=20) for 90 days. Full clinical history and fasting blood samples were obtained to determine FBG , HbA1c, FSI, full lipid profile, MDA , hs-CRP levels as well as HOMA-IR at the beginning and at the end of the study.

Results: Baseline and after 12 week FBG,HbA1c,FSI,TC,TG,LDL-c,VLDL-c, MDA, hs-CRP and HOMA-IR showed a highly significant difference among the 2 groups (p value < 0.001) . HDL-c level showed a significant difference in percentage of change (p value=0.009) among the study groups.

Conclusion: Administration of 140 mg of silymarin thrice daily over 90 days showed a superior efficacy than standard treatment alone.

INTRODUCTION

Diabetes has been defined as "a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both". The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessel**(ADA, 2015)**.

The International Diabetes Federation (IDF) estimates that 382 million people, 8.3% of the world population, suffered from diabetes in 2013 and this prevalence will increase to 592 million people or one adult in 10 will have diabetes by 2035. This equates to approximately three new cases every 10 seconds**(Tao et al., 2015)**. Regarding Egyptian society, nearly 10.4% of the Egyptian population (aged 10 - 79 years) has diabetes**(Soliman, 2013)**. It has been estimated to be the sixth most important cause of disability burden in Egypt**(Arafa and Amin, 2010)**.

The most common form of diabetes, type 2 diabetes mellitus (T2DM), is a multifactorial disease, the pathophysiology of which involves not only the pancreas but also the liver, skeletal muscle, adipose tissue, gastrointestinal tract, brain, and kidney. Reduced sensitivity to insulin (i.e., impaired insulin-mediated glucose disposal or insulin resistance) in liver, muscle, and adipose tissue, and a progressive decline in pancreatic β-cell function leading to impaired insulin secretion, eventually result in hyperglycemia, the hallmark feature of T2DM**(Cornell, 2015)**. Since inflammation and oxidative stress contribute to insulin resistance, lipid peroxidation and cardiovascular diseases, reduction of inflammatory biomarkers, oxidative stress indicators and hyperlipidaemia can help to better control diabetes**(Ebrahimpour Koujan et al., 2015)**.

The current standard of care for patients with DM include non-pharmacological treatment (caloric restriction and physical exercise), and pharmacological treatment (oral and injectable antidiabetic drugs) **(Pfeiffer and Klein, 2014)**.Because of the fact that a lack of highly effective drug-therapy with existing synthetic agents and their resulting adverse effects motivated further search into traditional medicine in order to find new natural entities to be used as anti-diabetic products**(Salimifar et al., 2013)**. The favorable effect of antioxidants in the treatment of oxidative metabolic derangement in diabetes has been reported in several experimental studies **(Fenercioglu et al., 2010; Tabatabaei-Malazy et al., 2014)**.

Silymarin, the active component of the milk thistle plant (*Silybum marianum (L.) Gaertn.*) is a polyphenolic flavonolignan with potentially antioxidant properties that comprises four flavonolignans isomers: silybin, isosilybin, silydianin , silychristin and one flavonoid, taxifolin **(Hackett et al., 2013)**. Silymarin and its components display diverse biological activities in vitro and in vivo, including antioxidant and anti-inflammatory properties. These biological activities are supposed to be the basis for the therapeutic potential of silymarin in diabetes **(Kazazis et al., 2014)**.
It has also several beneficial properties on a wide variety of other disorders such as renoprotective, hypolipidemic , anti-atherosclerotic, osteoprotective and anti-cancer activities .It can be used also in Alzheimer prevention, sepsis and burns treatment**(Karimi et al., 2011; Bahmani et al., 2015)** .

The aims of this study were to evaluate the efficacy of silymarin as adjuvant therapy to standard antidiabetic treatment on glycemic control, lipid profile and insulin resistance compared to standard antidiabetic treatment alone in patients with type 2 diabetes mellitus.

Patients and methods

The current study was Prospective, Randomized, and Placebo- Controlled, Single -Blinded, Pilot Study, conducted on 40 Egyptian adult outpatients with type 2 diabetes mellitus. The study was conducted at Internal Medicine Department, Ain Shams University Hospitals, Cairo, Egypt. The study protocol was revised and approved by the research ethics committee at Faculty of Pharmacy, Ain shams University. Prior to participation all eligible patients were educated about the study protocol and signed the written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Patients

Inclusion criteria comprised: Adults aged between 40 and 65 years, Previous diagnosis of Type 2 Diabetes according to American Diabetes Association criteria (ADA) from at least 6 months and Patients with suboptimal glycemic control despite the prescription of a diet, physical exercise, and hypoglycemic drugs. Patients were excluded from the study if they have; Severe liver disorders, renal insufficiency (serum creatinine > 2 mg/dl) ,Severe heart dysfunction (class III or higher according to the New York Heart Association classification), History of myocardial infarction or stroke, malignancy, psychiatric disease, Severe infections, Breastfeeding or pregnancy, Taking other herbal or multivitamin –mineral supplements, Smoking, corticosteroids use during the 2 months preceding the study, experienced any changes in their medications during the study period , use of P-glycoprotein antagonists.

Methods

The study included a total of 40 patients that were randomly assigned by simple randomization to one of 2 groups: Silymarin group (20 patients who received 140 mg silymarin capsules **Legalon®**, MADAUS GmbH, Cologne, Germany three times daily with their standard antidiabetic treatment for 90 days) and Placebo Control group (20 patients who received identical placebo capsules three times daily with their standard antidiabetic treatment for 90 days).

At baseline, all patients underwent thorough history taking and clinical examination. Blood samples were collected to measure the following parameters at baseline and at end of treatment and used to evaluate the treatment outcomes : Fasting Blood Glucose (FBG) , Glycated Hemoglobin (HbA1c), Fasting Serum Insulin (FSI), Total Cholesterol (TC) , Triglycerides (TG) , High Density Lipoprotein (HDL-C), Low Density Lipoprotein (LDL-C), Very Low Density Lipoprotein (VLDL-C), Malondialdehyde (MDA) , high sensitive C-Reactive Protein (hs-CRP) levels as well as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated from FBG and FSI values of patients using the following formula:
HOMA-IR = FPG (mg/dL) × FSI (μU/mL)/405.

Commercial kits using reliable spectrophotometric methods were used to measure FBG, HbA1c, TC, TG, HDL-C (Stanbio Laboratory,USA), MDA (Bio Diagnostic Laboratories, Egypt),while FSI(Chemux Bioscience, USA) and hs-CRP (DRG International , Inc.,USA) were measured by commercial kit with enzyme-linked immunosorbent assay method.

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 21. All p-values are two-sided. P-values < 0.05 were considered significant.

Results

From January 2014 to April 2015 a total of 78 patients were assessed for eligibility and only 57 diabetic patients fulfilled the inclusion criteria and were included in the study. However, Out of the 57 patients, only 40 completed the study. Seventeen patients were dropped out due to non-compliance or experienced changes in their medications within the first few days from their recruitment. Hence, per protocol statistical analysis was done.
Twenty females (50%) and twenty males (50%) were enrolled and distributed in the 2 groups as follows: 6 (30%) male and 14 (70%) female patients in Control group while 4 (20%) male and 16 (80%) female patients in Silymarin group. Patients' age ranged between 41-65 years. There was no significant difference among the 2 groups regarding age , gender, family history, education, employment, duration of diabetes, weight, height and body mass index(BMI) (p-value > 0.05) **(Table 1)**.

TABLE 1: BASELINE DEMOGRAPHICS AND CLINICAL CHARACTERISTICS

Parameter		Control Group (n=20)	Silymarin Group (n=20)	*P*-Value
Gender	Male	6 (30%)	4(20%)	0.465 [a]
	Female	14(70%)	16(80%)	
Family History	Positive	14(70%)	12(60%)	0.507 [a]
	Negative	6(30%)	8(40%)	
Education	Yes	12(60%)	12(60%)	1.000 [a]

	No	8(40%)	8(40%)	
Employment	Yes	11(55%)	10(50%)	0.752 [a]
	No	9(45%)	10(50%)	
Age(years**)** Median(Range)		51.0 (41.0-65.0)	51.5 (42.0-64.0)	0.841 [b]
Duration of Diabetes(years**)** Median(Range)		7.5 (2.0-20.0)	7.0 (2.0-20.0)	0.841 [b]
Weight(Kg) Median(Range)		89.0 (56.0-107.0)	87.5 (63.0-105.0)	1.000 [b]
Height(M) Median(Range)		1.58 (1.47-1.74)	1.56 (1.44-1.75)	0.547 [b]
BMI(Kg/M^2) Median(Range)		34.7 (25.9-46.8)	34.2 (28.8-43.3)	1.000 [b]

n=number of subjects.
Data are expressed as medians (inter quartile range) and numbers (%).
Statistical test: [a]: Chi-square test.
[b]: Mann-Whitney test.
Statistical significance was set at $p \leq 0.05$.
Control Group: Group receiving standard anti- diabetic treatment.
Silymarin Group: Group receiving standard anti- diabetic treatment and silymarin.

Clinical outcomes evaluation:

Efficacy

Baseline and after 3 months FBG,HbA1c and FSI levels for the 2 groups showed a highly significant difference (p value < 0.001) **(Table 2)**.Also there was a highly significant difference in HOMA-IR (p value < 0.001) among the 2 groups **(Figure1)**.

Baseline and after 3 months TC, TG, LDL-C and VLDL-C levels for the 2 groups showed a highly significant difference (p value < 0.001) **(Table 3)**.However there was no significant difference in HDL-C level among the 2 groups but it showed a significant decrease in percentage of change (p value=0.009) among the 2 groups **(Figure2)**.

Regarding MDA and hs-CRP levels there was a highly significant difference (p value < 0.001) among the 2 groups for both **(Table 4)**.

TABLE 2: COMPARISON OF GLYCEMIC RELATED PARAMETERS AT BASELINE AND AFTER 3 MONTHS AMONG THE STUDY GROUPS

Parameter	Control Group (n=20)	Silymarin Group (n=20)	***p*-Value 1**
HbA1c (%)			

at Baseline After 3 months	9.4 (8.2-12.1) 10.7 (7.5-11.9)	10.4 (8.0-12.3) 8.5 (6.3-12.3)	0.884 [a] <0.001 [a]*
***p*-Value 2**	0.023[b]*	0.001 [b]*	
FBG(mg/dl) at Baseline After 3 months	 202.5 (152.0-312.0) 252.0 (141.0-370.0)	 252.5 (174.0-395.0) 162.0 (109.0-391.0)	 0.112 [a] <0.001 [a]*
***p*-Value 2**	0.089 [b]	0.003 [b]*	
FSI(µIU/mL) at Baseline After 3 months	 15.1 (10.1-23.8) 19.7 (9.4-24.4)	 15.2 (8.4-20.7) 11.2 (9.3-15.6)	 1.000 [a] <0.001 [a]*
***p*-Value 2**	0.002 [b]*	0.001 [b]*	

n=number of subject.
Data are expressed as medians (inter quartile range).
Statistical Test: [a]: Mann-Whitney test.
[b]:Wilcoxon Signed Rank test.
P-value1 = P-value for the comparisosns between the groups.
P-value2 = P-value for the change with time within each group.
*:Significant difference was set at $p \leq 0.05$.
Control Group: Group receiving standard anti- diabetic treatment.
Silymarin Group: Group receiving standard anti- diabetic treatment and silymarin.

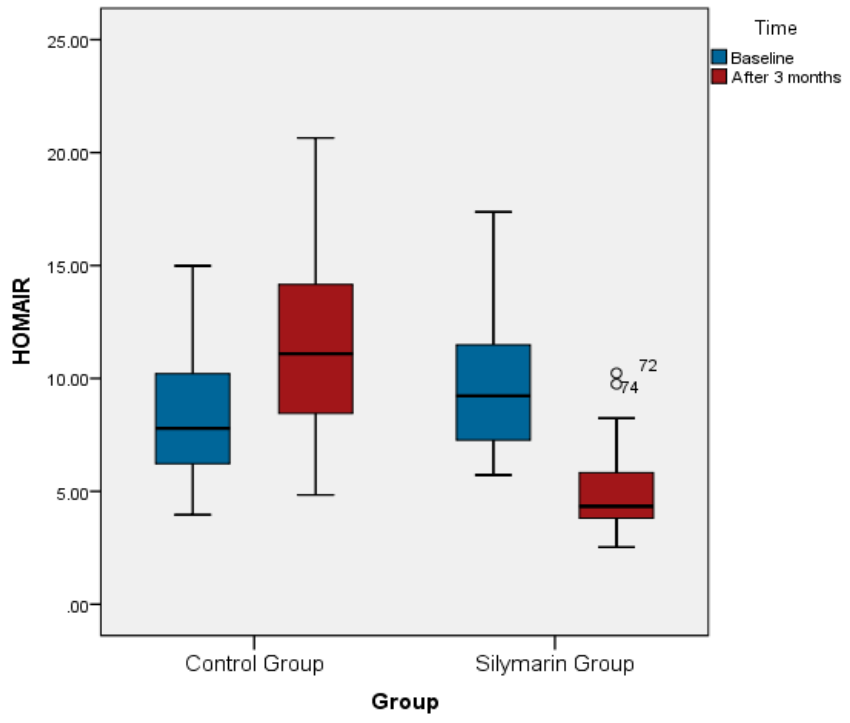

FIGURE 1: HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE AT BASELINE AND AFTER 3 MONTHS FOR THE STUDY GROUPS

TABLE 3: COMPARISON OF LIPID PROFILE PARAMETERS AT BASELINE AND AFTER 3 MONTHS AMONG THE STUDY GROUPS

Parameter	Control Group (n=20)	Silymarin Group (n=20)	***P*-Value 1**
TC(mg/dl)			
at Baseline	183.5 (132.0-260.0)	187.0 (116.0-280.0)	1.000 [a]
After 3 months	219.5 (136.0-297.0)	155.5 (113.0-213.0)	<0.001 [a]*
***P*-Value 2**	0.017 [b]*	0.010 [b]*	
TG(mg/dl)			
at Baseline	165.5 (92.0-245.0)	171.5 (95.0-235.0)	1.000 [a]
After 3 months	194.5 (116.0-289.0)	104.0 (83.0-175.0)	<0.001 [a]*
***P*-Value 2**	0.007 [b]*	0.001 [b]*	
HDL-C(mg/dl)			
at Baseline	36.5 (14.0-53.0)	23 (12.0-52.0)	0.096 [a]
After 3 months	33.5 (19.0-46.0)	38.5 (14.0-65.0)	0.132 [a]
***P*-Value 2**	0.558 [b]	0.004 [b]*	
LDL-C(mg/dl)			
at Baseline	122.5 (62.0-192.0)	131.9 (69.0-218.6)	1.000 [a]
After 3 months	154.8 (66.2-220.2)	94.0 (58.8-154.2)	<0.001 [a]*
***P*-Value 2**	0.028 [b]*	0.005 [b]*	
VLDL-C(mg/dl)			
at Baseline	33.1 (18.4-49.0)	34.3 (19.0-47.0)	1.000 [a]
After 3 months	38.9 (23.2-5.8)	20.8 (16.6-35.0)	<0.001 [a]*
***P*-Value 2**	0.007 [b]*	0.001 [b]*	

n=number of subjects.
Data are expressed as medians (inter quartile range).
Statistical Test: [a]: Mann-Whitney test.
[b]:Wilcoxon Signed Rank test.
P-value1 = P-value for the comparisosns between the groups.
P-value2 = P-value for the change with time within each group.
*:Significant difference was set at $p \leq 0.05$.
Control Group: Group receiving standard anti- diabetic treatment.
Silymarin Group: Group receiving standard anti- diabetic treatment and silymarin.

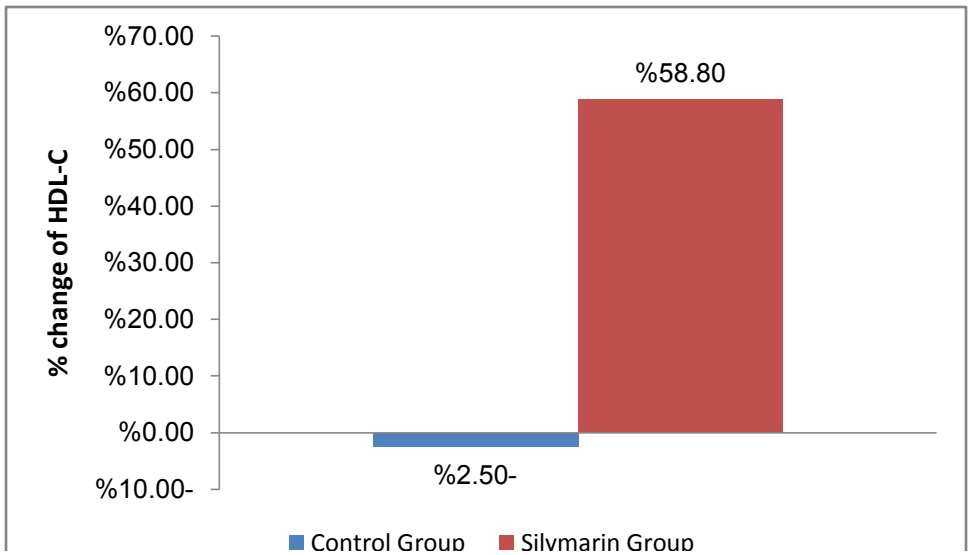

FIGURE 2: HIGH DENSITY LIPOPROTEIN CHOLESTEROL PERCENTAGE OF CHANGE FOR THE STUDY GROUPS

TABLE 4: COMPARISON OF SERUM MALONDIALDEHYDE AND C-REACTIVE PROTEIN LEVELS AT BASELINE AND AFTER 3 MONTHS AMONG THE STUDY GROUPS

Parameter	Control Group (n=20)	Silymarin Group (n=20)	***p*-Value 1**
MDA(nmol/mL**)**			
at Baseline	37.6 (20.3-46.2)	39.0 (25.0-58.8)	1.000 [a]
After 3 months	40.1 (20.8-66.4)	28.0 (17.3-45.7)	<0.001 [a]*
***p*-Value 2**	0.020 [b]*	<0.001 [b]*	
CRP(mg/L)			
at Baseline	3.6 (2.2-5.6)	4.2 (2.9-5.6)	1.000 [a]
After 3 months	4.6 (1.2-6.6)	2.7 (1.2-6.1)	<0.001 [a]*
***p*-Value 2**	0.030 [b]*	0.001 [b]*	

n=number of subjects.
Data are expressed as medians (inter quartile range).
Statistical Test: [a]: Mann-Whitney test.
[b]:Wilcoxon Signed Rank test.
P-value1 = P-value for the comparisosns between the groups.
P-value2 = P-value for the change with time within each group.
*:Significant difference was set at $p \leq 0.05$.
Control Group: Group receiving standard anti- diabetic treatment.
Silymarin Group: Group receiving standard anti- diabetic treatment and silymarin.

Discussion

Several mechanisms have been proposed to explain why is silymarin suggested as an attractive candidate for the treatment of diabetes and its complications. Numerous experimental studies have documented that silymarin and its components display diverse biological activities in vitro and in vivo, including antioxidant, anti-inflammatory, membrane-stabilizing, inhibition of gluconeogenesis, peroxisome proliferator-activated receptor γ (PPARγ) agonist properties, increase insulin gene expression as well as beta-cell proliferation. These biological activities are supposed to be the basis for the therapeutic potential of silymarin in diabetes**(Guigas et al., 2007; Detaille et al., 2008; Pferschy-Wenzig et al., 2014; Soto et al., 2014)**.

Since that, we conducted the present study as a clinical trial to investigate the effect of silymarin supplementation on serum glucose , lipid as well as insulin resistance in Egyptian type 2 diabetic patients. These experimental studies support the findings of our study.

The primary finding of this study suggested that consuming 140 mg silymarin supplement thrice daily for 90 days modulates glycemic and insulin resistance markers. Silymarin significantly decreased FBG, HbA1c compared to placebo controlled group. Also FSI and HOMA-IR significantly decreased in silymarin group in comparison to control group which indicated that alleviation of insulin resistance due to silymarin treatment.

Our findings were in accord with the results of Huseini et.al whose supplementation of type II diabetes patients with 200 mg silymarin thrice daily for 120 days led to a significant decrease in HbA1c and FBS levels in silymarin treated patients compared with placebo as well as with values at the beginning of the study in each group**(Huseini et al., 2006)**.Hussain et al. in a clinical trial of type 2 diabetic patients who present with poor response to glibenclamide, showed that 200 mg/day silymarin for 120 days significantly reduced fasting plasma glucose excursion , in addition to significantly reducing HbA1c levels**(Hussain, 2007)**. In 1997 Velussi et al. conducted a 12 month clinical trial in alcoholic liver cirrhosis patients with diabetes who received 600 mg of silymarin daily, results indicated that silymarin can reduce FBG, HbA1c and fasting blood insulin **(Velussi et al., 1997)**.

Diabetes produces disturbances in lipid profiles, especially an increased susceptibility to lipid peroxidation, which is responsible for the increased incidence of atherosclerosis, a major complication of diabetes mellitus**(Moussa, 2008)**.

It has been reported previously that silymarin or its polyphenolic fraction modifies the lipoprotein profile in animal model of dyslipidaemia**(Radjabian and Huseini, 2010; Sajedianfard et al., 2014)**. Positive effect of silymarin on total plasma cholesterol is partly caused by higher elimination of the cholesterol mediated by CYP7A1, very important enzyme involved in cholesterol elimination, and the increase of protein expression of the necessary transporters for the cholesterol efflux from the hepatocytes into the bile**(Poruba et al., 2015)**. The decreased plasma level of triglycerides could by caused by increased triglycerides metabolism. It has been reported that treatment with silymarin improves LDL- binding to the hepatocytes, an important factor in the reduction of plasma LDL-C levels through clearance by hepatocytes. Elevation of HDL-C levels in the serum after silymarin treatment can be explained by the increase in the production and secretion of apo– AI by the liver, which is considered as the main component of apo lipoproteins of HDL**(Mesheimish et al., 2007)**.
In our study consuming 140 mg silymarin supplement thrice daily for 90 days significantly decreased TC, TG, LDL-C and VLDL-C levels in silymarin group in comparison to placebo control group . Also percentage of change of HDL-C was significantly increased between the two groups.
Our findings were in accord with the results of Mesheimish et al. whose supplementation of patients with dyslipidaemia of various etiologies with 400mg / day silymarin for 60 days showed that treatment with silymarin resulted in a significant decrease in TC, TG, LDL-C and VLDL-C levels, with a significant elevation in HDL-C levels**(Mesheimish et al., 2007)**.Also our results were in agreement with the results of Huseini et.al as silymarin treatment significantly lowered blood levels of TC, TG,LDL and significantly increased HDL**(Huseini et al., 2006)**.

Free radical damage induced by oxidant agents or hyperglycemia play a direct role in the initiation of oxidative stress, the accumulation of lipid peroxidation products and the formation of advanced glycation end products and inflammation. Silymarin has shown protective effect against oxidative lipid peroxidation in several experimental models and in human hepatic damage through the scavenging of free radicals **(Soto et al., 2003; Roozbeh et al., 2011)**.
In our study silymarin significantly decreased MDA and hs-CRP levels in silymarin group in comparison to placebo control group. Our results were in agreement with the results of Ebrahimpour et al. in which 40 type 2 diabetic patients received 140 mg silymarin thrice daily for 45 days. There was a significant reduction in hs-CRP and MDA levels in the silymarin group compared to the placebo group **(Ebrahimpour Koujan et al., 2015)**.

Conclusion

Administration of 140 mg of Silymarin thrice daily over 90 days showed a superior efficacy than standard treatment alone. It resulted in significant decrease in serum levels of FBG, HbA1c, FSI, TC, TG, LDL-C, VLDL-C, MDA and hs-CRP. Moreover, silymarin was superior to standard treatment alone in improving HOMA-IR and insulin resistance.

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None of the authors have any conflict of interest to disclose.

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