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Effects of *Silybum marianum (L.) Gaertn.* (silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: A randomized, triple-blind, placebo-controlled clinical trial

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ABSTRACT

Aim: Diabetes is a serious metabolic disorder and oxidative stress and inflammation contribute to its pathogenesis and complications. Since *Silybum marianum (L.) Gaertn.* (silymarin) extract is an antioxidant with anti-inflammatory properties, this randomized clinical trial was conducted to evaluate the effects of silymarin supplementation on oxidative stress indices and hs-CRP in type 2 diabetes mellitus patients.
Methods: For the present paralleled, randomized, triple-blinded, placebo-controlled clinical trial, 40 type 2 diabetes patients aged 25–50 yr old and on stable medication were recruited from the Iranian Diabetes Society and endocrinology clinics in East Azarbayjan (Tabriz, Iran) and randomly assigned into two groups. Patients in the silymarin treatment group received 140 mg, thrice daily of dried extracts of *Silybum marianum* ($n = 20$) and those in the placebo group ($n = 20$) received identical placebos for 45 days. Data pertaining to height, weight, waist circumference and BMI, as well as food consumption, were collected at base line and at the conclusion of the study. Fasting blood samples were obtained and antioxidant indices and hs-CRP were assessed at baseline, as well as at the end of the trial.
Results: All 40 patients completed the study and did not report any adverse effects or symptoms with the silymarin supplementation. Silymarin supplementation significantly increased superoxide dismutase (SOD), glutathione peroxidase (GPX) activity and total antioxidant capacity (TAC) compared to patients taking the placebo, by 12.85%, 30.32% and 8.43%, respectively ($p < 0.05$). There was a significant reduction in hs-CRP levels by 26.83% ($p < 0.05$) in the silymarin group compared to the placebo group. Malondialdehyde (MDA) concentration significantly decreased by 12.01% ($p < 0.05$) in the silymarin group compared to the baseline.
Conclusions: Silymarin supplementation improves some antioxidant indices (SOD, GPX and TAC) and decrease hs-CRP levels in T2DM patients.

Abbreviations

ANCOVA	Analysis of covariance
BMI	Body mass index
CAT	Catalase
FBS	Fasting blood sugar
GSHPx	Glutathione peroxidase
HOMA	Homeostasis model assessment
hs-CRP	High-sensitivity C-reactive protein
IkBα	Inhibitor of nuclear factor kappa B (NF-κB) alpha
IL-1β	Interleukin-1 beta
IL-2	Interleukin 2
INF-γ	Interferon gamma
IPAQ	International physical activity questionnaire
JNK	c-Jun N-terminal kinases
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MEK	Mitogen-activated protein kinase kinase
NAFLD	Non-alcoholic fatty liver disease

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NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
OHAs	Oral hypoglycaemic agents
PAL	Physical activity levels
PGE2	Prostaglandin E2
PKC	Protein kinase C
RAS	Random allocation software
RBC	Red blood cell
SOD	Superoxide dismutase
T2DM	Type 2 diabetes mellitus
TAC	Total antioxidant capacity
TGF-β1	Transforming growth factor-β1
TNF-α	Tumor necrosis factor-α
WC	Waist circumference

Introduction

As a complex metabolic condition, diabetes mellitus is a risk factor for coronary artery diseases that affect both individuals and society as a whole. It is estimated that in 2030, 522 million adults will be suffering from diabetes worldwide (IDF 2011). Cardiovascular diseases are the main causes of mortality in type 2 diabetes mellitus (T2DM) patients. Pathophysiological conditions associated with T2DM and coronary artery outcomes are inflammation and oxidative stress (Pooya et al. 2010). Oxidative stress is defined as an imbalance between the amount of oxidants produced and the antioxidant defence system. Antioxidant vitamins and antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPX) results in increased blood glucose and consequently insulin levels, one of the mechanisms assumed to lead to the onset and progression of diabetes complications (Rains and Jain 2011). Additionally, oxidative damage by free radicals and highly toxic products formed by the above mentioned process, such as Malondialdehyde (MDA) and lipid peroxidation products potentially cause insulin resistance (Rains and Jain 2011). Furthermore, some studies have indicated that oxidative stress and insulin resistance are associated with higher levels of C-reactive protein and other biomarkers of subclinical systemic inflammation (Garcia et al. 2010). 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 (Pooya et al. 2010; Garcia et al. 2010). Although poor glycaemic control is related to T2DM complications, adherence to oral hypoglycaemic agents (OHAs) is low, which may be due to their various side effects (Khan and Siddiqui 2010).

Recently, more attention has been paid to natural herbal combination remedies in attempt to decrease type 2 diabetes complications, especially because of their lesser adverse side effects, lower costs and the better health benefits of these therapies, as well as their acceptability among people (Khan and Siddiqui 2010). Silymarin, the active component of the milk thistle plant (*Silybum marianum (L.) Gaertn.*) (Russell 2004) is a polyphenolic flavonolignan with potentially antioxidant properties that comprises four flavonolignans isomers: silybin, isosilybin, silydianin and silychristin (Roozbeh et al. 2011). Silymarin has shown protective effects against oxidative lipid peroxidation in several experimental models and in human hepatic damage through the scavenging of free radical and increasing reduced glutathione (GSH) (Wellington and Harvis 2001). Silymarin in rats with alloxan-induced diabetes mellitus significantly increase the activity of antioxidant enzymes such as SOD, GPX and catalase (CAT), and prevents renal tissue damage (Soto et al. 2003, 2010). Oral supplementation of end stage renal disease patients with Silybum marianum (Livergol®) extract and vitamin E led to a reduction of MDA and increases in red blood cell (RBC) GPX levels (Roozbeh et al. 2011). Regarding the above-mentioned experimental studies (Soto et al. 2003, 2010; Wellington and Harvis 2001), silymarin potentially has antioxidant and anti-inflammatory characteristics; however, studies investigating the effects of silymarin on stress oxidative and inflammatory biomarkers have been limited to animal models and no clinical trials have been performed on diabetic patients. Therefore, we conducted the present study as the first clinical trial for determining the effects of Silybum marianum extract (silymarin) on total antioxidant capacity (TAC), SOD, GPX, MDA and hs-CRP in T2DM patients.

Materials and methods

Study design

The target population of the present study was T2DM patients who were recruited from the Iranian Diabetes Society in East Azarbayjan (Tabriz, Iran) and from endocrinology and metabolism clinics associated with Tabriz University of Medical Sciences, Tabriz, Iran. Forty T2DM patients (20 male and 20 female), 25–50 yr of age who had been diagnosed with diabetes for at least six months prior to enrolment in the study participated in the study. A patient had to meet the following criteria to be included in the study: diagnosis of type 2 diabetes based on World Health Organization guidelines (FBS >126 mg/dl or 2HP > 200 mg/dl or HbA1c >9%) (Consultation 1999), taking hypoglycaemic medications, having a body mass index (BMI) between 27 and 35 kg/m^2 and following a stable habitual diet for the past three months. Exclusion criteria was defined as: taking herbal or antioxidant and multivitamin-mineral supplements, insulin therapy, being pregnant, lactating, suffering from cancer, gastrointestinal disease, autoimmune and inflammatory diseases, smoking, receiving drugs that interact with blood glucose, oxidative stress and inflammatory markers, and having experienced any changes in their medications in the previous two months and/or during the study period. Sample size was determined based on data from a previous study (Huseini et al. 2006). By considering the (mean ± SD) for serum HDL-c concentration, a confidence interval of 95%, $\alpha = 0.05$ and power of 80%, 18 T2DM patients were computed per group. Taking into account a possible loss of 15% of the subjects in the follow-up period, as well as those who may not complete the study protocol, 20 patients were finally allocated to each group.

The present work was conducted as a randomized, parallel, placebo-controlled, triple-blind study undertaken at the Tabriz University of Medical Sciences. After recruiting 56 T2DM patients, the individual questionnaire, including demographic characteristics, disease history and medications, was completed for each patient through comprehensive interviews. Forty eligible subjects were randomly divided into two groups using a block randomization procedure (Random Allocation Software: RAS) (Saghaei 2004) (of size 4) which matched patients into the two groups based on BMI and sex. A diagram of the study design is shown in Fig. 1. The present study was conducted in accordance with the guidelines laid down in the Declaration of Helsinki (1964), and all procedures involving human persons were approved by the ethics committee of Tabriz University of Medical Sciences (no. 9289). Written informed consent was obtained from all subjects and the study was registered on the Iranian Registry of Clinical Trials website (available at: http://www.irct.ir, identifier: IRCT201309243140N13).

All patients, the researchers, endocrinologist, statistical analyst and the laboratory staff were blinded to the intervention. According to the findings of previous studies, the supplementation procedure for silymarin was set at three 140 milligrams (mg) tablets per day (Roozbeh et al. 2011). The experimental group received 140 mg silymarin supplement three times daily with main meals for 45 days. Each 140 mg coated tablet of silymarin supplement contained the dried extracts of *Silybum marianum* (extracted with acetone and water) equivalent to 140 mg silymarin (Livergol®) (Goldaru Herbal Products Pharmaceutical Company, Isfahan, Iran). The placebo group were given a similar amount of placebo tablets containing lactose three times daily

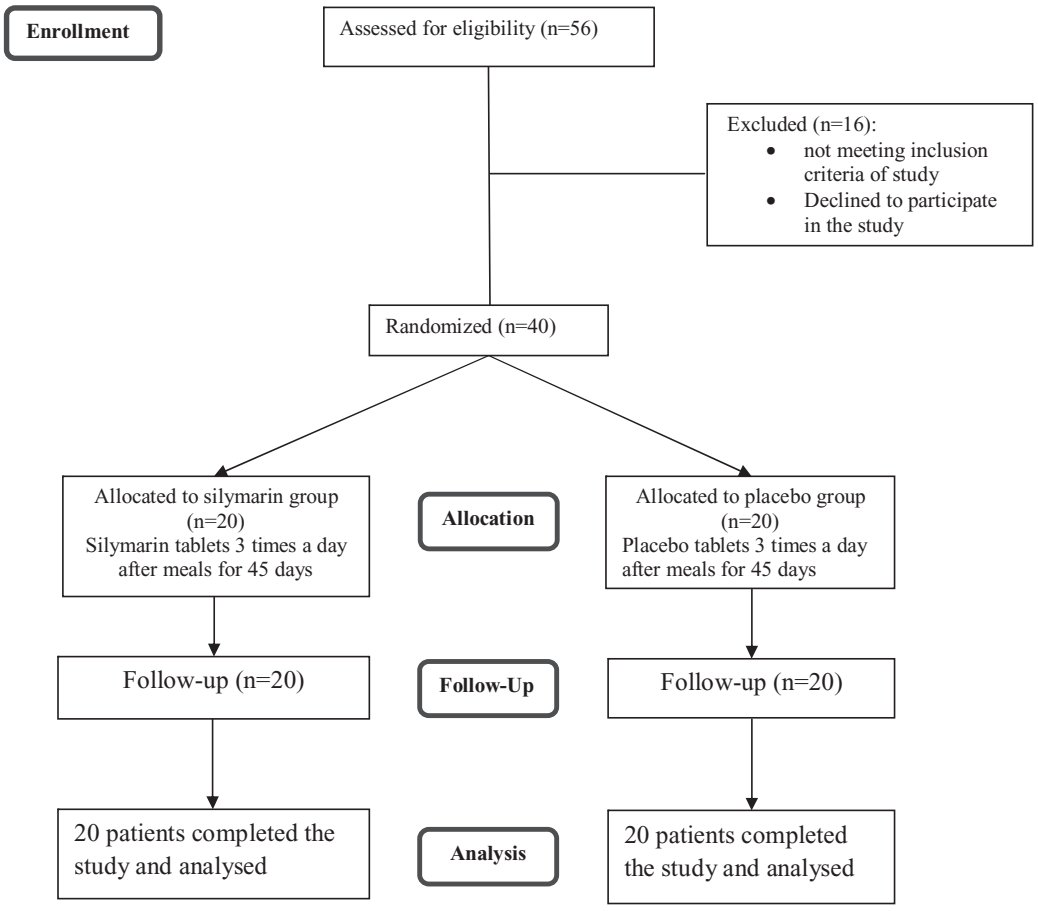

Fig. 1. Diagram of the study design.

with main meals for 45 days. Placebo tablets were kindly produced by the Pharmaceutical faculty of Tabriz University of Medical Sciences. A third person who was not directly involved in the study was responsible for packaging the silymarin and placebo tablets in bottles that were coded as 1 or 2. The code descriptors remained unknown to the researcher until the end of the study. Patients were given their bottles upon their first visit to Sheykholrayis Polyclinic after a one week run-out period. To determine the two medication groups' adherence, as well as to follow the dropout rate and to ensure the consumption of the supplements, a check list was designed and the subjects were asked to sign it each time they took the supplement. Compliance to this request was assessed indirectly by counting the remaining tablets in the bottles. Patients who consumed more than 90% of the tablets had good compliance and were included in statistical analyses.

Outcomes

The primary outcomes of the present clinical trial according to the aims of the study were the effects of Silybum marianum (L.) Gaertn. (silymarin) extract supplementation on concentrations of SOD, GPX, TAC, MDA and hs-CRP. The secondary outcomes were the effects of silymarin supplementation on daily energy, dietary nutrient intakes and anthropometric indices.

Anthropometric and dietary assessments

Data on anthropometric indices including body weight, height, waist circumference (WC) and BMI were obtained at the beginning and end of the trial. Body weight was measured in a fasting state, without shoes and wearing light clothing to the nearest 0.5 kg accuracy using a weighing calibrated scale (Seca, Hamburg, Germany). Height was measured by mounted tape, without shoes and at a standing position near to the wall to the nearest 0.1 cm accuracy using a stadiometer (Seca, Hamburg, Germany). WC was measured by non-stretching tape measure around the abdomen. BMI was calculated by dividing weight in kilogram by height in meters squared. Nutrient intake data for all subjects were collected using three-day diet records (one for the weekend and two for week days) at the start and at the end of the study. Dietary intakes were analyzed using the Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA, USA), modified for Iranian food.

Blood sampling and biochemical measurements

Prior to intervention and at the end of the trial, 8 ml overnight fasting venous blood samples were obtained from each patient by Venoject glass tubes between 7:00 and 9:00. Serum was separated by centrifugation at 2500 rpm for 10 min (Orum Tadjhiz Centrifuge, Iran) and immediately stored at −70 °C. Measurement of antioxidant indices, TAC in serum (Miller et al. 1993) and antioxidant enzymes, SOD and GSH-Px in red blood cells were performed using reliable spectrophotometric methods with commercial kits (TAC, RANDOX kits, SOD, RANSOD kits and GPX, RANSEL kits; RANDOX Laboratory, Crumlin, UK), on an automatic analyser (Abbott model Alcyon 300; Abbott Laboratories, Abbott Park, IL, USA). Serum MDA activities (used as a marker for oxidative stress) were determined through a reaction with thiobarbituric acid (TBA), a highly sensitive method (Richard 1992) in order to produce a pink-colored complex. The fluorescent intensity of the pink-colored complex was measured at 547 nm with an excitation at 525 nm using a spectrofluorometer (model SFM 25A; Kontron,

Table 1
General characteristics of T2DM patients at baseline.

Variable	Silymarin group ($n = 20$)	placbo group ($n = 20$)
Age (yr)	43.50 ± 5.76	46.10 ± 4.30
Male/Female *N* (%)	11(55)/9(45)	9(45)/11(55)
Duration of diabetes (yr)	5.00 ± 3.69	4.22 ± 2.57
Metformin 500 mg, tablets/day	2.50 ± 0.60	2.50 ± 0.51
Glibenclamide 5 mg, tablets/day	0.70 ± 0.86	0.80 ± 0.83
Height (cm)	168.00 ± 12.05	163.70 ± 9.67
Weight (kg)	86.35 ± 11.26	80.35 ± 16.13
BMI (kg/m^2)	30.77 ± 2.48	30.03 ± 4.49
WC (cm)	103.25 ± 8.39	100.85 ± 11.88

BMI: Body mass index; WC: waist circumference
Values are presented as mean ± standard deviation except sex that presented as number (percent). For all characteristics, there were no significant differences between the silymarin and placebo groups (all $P > 0.05$, based on independent samples *t*-tests for quantitative and chi-square for sex).

Milan, Italy). Quantification of serum hs-CRP levels was determined by biochemical analysis using the immunoturbidimetry assay via a Biosystem CPR-hs kit (Biosystem, SA, Barcelona, Spain).

Statistical analyses

Statistical analyses of all data were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Data results were expressed as mean ± standard deviation (±SD). The one-sample Kolmogorov–Smirnov test was used for assessing the normality of the distribution of data. Baseline variables in the two groups were compared using an independent sample *t*-test and chi-square test for quantitative and qualitative variables, respectively. A paired sample *t*-test was employed for means comparisons of all variables in order to identify within-group differences. Analysis of covariance (ANCOVA) was used to identify any differences between the two groups after intervention, adjusting the results for the baseline measurements and covariates. The percentage of changes in variables following the intervention was also determined:

[(after values–before values)/before values]
$\times 100$ and reported as mean.

$p < 0.05$ was considered statistically significant.

Results

All 40 patients, including the 20 patients in the silymarin group and 20 patients in the placebo group completed the 45-day clinical trial. Capsule counts showed that all patients who completed the study had acceptable compliance with the intervention and did not report any adverse effects or symptoms with the silymarin supplementation. Table 1 shows that there were no significant differences between the groups in terms of the baseline values of demographic characteristics, nor within the anthropometric measures of participants ($p > 0.05$).

Effects of silymarin supplementation on daily energy and dietary nutrient intakes

Table 2 shows daily energy and dietary nutrient intakes of T2DM patients at the baseline and after 45 days of silymarin intervention. The comparisons within groups revealed that there were no significant changes in daily energy and dietary nutrients intakes for both the silymarin and placebo groups. Therefore, dietary factors did not confound the results for oxidative stress and inflammatory biomarkers; lipid profile levels, as well as all changes in biochemical measures by the end of the trial were attributed to silymarin antioxidant properties.

Effects of silymarin supplementation on serum oxidative stress parameters and hs-CRP

The SOD, GPX, TAC, MDA and hs-CRP concentrations before and after the intervention are shown in Table 3. At the start of the study, there were no significant differences between the silymarin group and the placebo group for TAC, SOD, MDA and hs-CRP concentrations ($p > 0.05$), whereas the two groups showed a significant difference in pretreatment levels of GPX ($p = 0.021$). After 45 days, the supplementation of T2DM patients with silymarin led to a significant increase in SOD, GPX and TAC by 12.85% (vs. a 10.55% reduction in the placebo group), 30.32% (vs. a 21.90% reduction in the placebo group) and 8.43% (vs. a 0.05% increase in the placebo group), respectively. Additionally, there was a significant decrease in hs-CRP concentration (by 26.38%) in the silymarin group (vs. a 124.24% increase in the placebo group) by the end of the study. Moreover, a significant 12.01% decrease in MDA levels (vs. a 15.37% increase in the placebo group) was observed in the silymarin group compared to baseline values. Silymarin supplementation caused a significant increase in SOD ($p = 0.003$), GPX ($p = 0.002$) and TAC ($p = 0.011$), and a significant reduction in hs-CRP ($p = 0.019$) levels compared to the placebo group. (ANCOVA adjusted for energy intake changes, weight changes, physical activity changes, intakes of OHAs, duration of diabetes and baseline values). MDA concentration had significantly decreased in the silymarin group by the end of the study (49.00 ± 17.52 to 42.40 ± 18.75, $p = 0.038$, paired *t*-test). After adjusting for energy intake changes, weight changes, physical activity change, intakes of OHAs, duration of diabetes and baseline values, no significant differences were found between the two groups compared with the placebo group ($p = 0.222$).

Discussion

Since silymarin potentially exhibits antioxidant and anti-inflammatory effects, we conducted the present study as the first clinical trial to investigate the effect of silymarin supplementation on serum oxidative stress parameters and hs-CRP in type 2 diabetic patients. The primary findings of this study suggested that consuming 140 mg silymarin supplement thrice daily for 45 days modulates oxidative stress and inflammatory biomarkers. Silymarin significantly enhanced SOD and GPX levels and decreased hs-CRP compared to the control. TAC levels as an indicator of the overall protective effect of antioxidants in body fluids, on cell membranes and other components of cells against oxidative damage (Koca et al. 2011) were elevated in the silymarin supplementation group compared to the placebo. Additionally, a significant decrease was observed in serum levels of MDA in the silymarin group compared to the baseline. It was therefore concluded that silymarin may be beneficial in the management of diabetes complications.

Table 2
Dietary energy and nutrients intakes of T2DM patients at the baseline and after 45 days of intervention.

Variable	Measurement period	Silymarin group ($n = 20$)	Placebo group ($n = 20$)	MD(95%CI), *P* value
Energy (kcal/day)	Baseline	2267.60 ± 336.09	2265.25 ± 291.58	2.35(−199.06, 203.76), 0.981[a]
	After intervention	2271.40 ± 333.61	2237.75 ± 288.22	31.58(−61.83, 125.00), 0.498[c]
	MD(95%CI), *P* value[b]	3.80(−85.40, 93.00), 0.930	−27.50(−69.26, 14.26), 0.184	
Carbohydrate (g/day)	Baseline	309.96 ± 67.65	312.19 ± 69.51	−2.23(−46.14, 41.68), 0.919[a]
	After intervention	294.28 ± 55.08	298.71 ± 56.79	−3.82(−37.68, 30.04), 0.820[c]
	MD(95%CI), *P* value[b]	−15.73(−52.19, 20.73), 0.378	−13.48(−43.20, 16.24), 0.355	
Protein (g/day)	Baseline	78.26 ± 21.12	73.74 ± 21.20	4.51(−9.03, 18.06), 0.504[a]
	After intervention	85.10 ± 25.25	81.92 ± 22.17	0.50(−12.68, 13.70), 0.939[c]
	MD(95%CI), *P* value[b]	6.84(−0.35, 14.04), 0.061	8.17(−4.45, 20.81), 0.191	
Total fat (g/day)	Baseline	82.80 ± 22.08	83.63 ± 25.50	−0.82(−16.09, 14.44), 0.914[a]
	After intervention	85.58 ± 25.36	81.32 ± 21.23	4.46(−10.17, 19.11), 0.540[c]
	MD (95%CI), *P* value[b]	2.77(−11.09, 16.63), 0.680	−2.30(−15.23, 10.61), 0.713	
MUFA (g/day)	Baseline	22.64 ± 7.38	21.30 ± 7.55	1.34(−3.44, 6.12), 0.574[a]
	After intervention	23.84 ± 11.36	21.69 ± 7.65	1.86(−4.55, 8.29), 0.559[c]
	MD(95%CI), *P* value[b]	1.199(−5.51, 7.91), 0.712	0.39(−3.17, 3.96), 0.821	
PUFA (g/day)	Baseline	24.19 ± 11.31	25.45 ± 15.00	−1.26(−9.76, 7.24), 0.765[a]
	After intervention	21.29 ± 11.71	23.75 ± 8.94	−2.66(−9.29, 3.97), 0.422[c]
	MD(95%CI), *P* value[b]	−2.89(−10.35, 4.55), 0.426	−1.70(−11.42, 8.01), 0.718	
SFA (g/day)	Baseline	23.35 ± 8.76	21.68 ± 7.87	1.66(−3.66, 7.00), 0.531[a]
	After intervention	27.37 ± 27.50	23.05 ± 8.43	3.93(−4.94, 12.80), 0.375[c]
	MD(95%CI), *P* value[b]	4.02(−4.93, 12.98), 0.359	1.37(−2.94, 5.68), 0.514	
Fiber (g/day)	Baseline	15.86 ± 6.37	12.90 ± 5.59	2.96(−0.87, 6.80), 0.126[a]
	After intervention	13.76 ± 4.24	13.49 ± 6.55	0.21(−3.48, 3.90), 0.908[c]
	MD(95%CI), *P* value[b]	−2.10(−6.27, 2.07), 0.305	0.59(−2.73, 3.92), 0.712	
Vitamin E (mg/day)	Baseline	2.24 ± 1.36	2.81 ± 1.91	−0.56(−1.64, 0.50), 0.290[a]
	After intervention	2.40 ± 1.30	1.88 ± 1.16	0.57(−0.23, 1.38), 0.160[c]
	MD(95%CI), *P* value[b]	0.16(−0.61, 0.94), 0.665	−0.92(−1.95, 0.10), 0.076	
Vitamin C (mg/day)	Baseline	142.45 ± 100.81	118.45 ± 92.39	23.99(−37.90, 85.89), 0.437[a]
	After intervention	118.95 ± 77.22	98.52 ± 81.79	14.75(−35.11, 64.63), 0.552[c]
	MD(95%CI), *P* value[b]	−23.49(−68.62, 21.63), 0.289	−19.93(−73.72, 33.86), 0.448	
Selenium (mg/day)	Baseline	0.06 ± 0.02	0.05 ± 0.02	0.01(−0.003, 0.03), 0.120[a]
	After intervention	0.06 ± 0.03	0.06 ± 0.03	0.007(−0.01, 0.03), 0.546[c]
	MD(95%CI), *P* value[b]	0.003(−0.01, 0.02), 0.682	0.01(−0.009, 0.03), 0.299	

MUFA: mono unsaturated fatty acid; PUFA: poly unsaturated fatty acid; SFA: saturated fatty acid.
The results are described as mean ± standard deviation (SD). [a]MD(95%CI), *P* value is reported based on the analysis of independent sample *t*-test. [b]MD(95%CI), *P* value is reported based on the analysis of paired sample *t*-test. [c]MD(95%CI), *P* value is reported based on the analysis of covariance.

Table 3
Serum oxidative stress parameters and hs-CRP of T2DM patients at the baseline and after 45 days of silymarin intervention.

Variable	Measurement period	Silymarin group ($n = 20$)	Placebo group ($n = 20$)	MD(95% CI), *P* value
SOD (U/g Hb)	Baseline	1373.03 ± 188.83	1559.30 ± 376.93	−186.26(−377.11, 4.57), 0.055[a]
	After intervention	1542.82 ± 219.92	1398.40 ± 351.78	250.46(92.92, 407.99), 0.003[c]
	MD(95%CI), *P* value[b]	169.79(96.42, 243.16), <0.001	−160.90(−235.58, −86.22), <0.001	
GPX (U/g Hb)	Baseline	19.40 ± 4.65	27.44 ± 14.17	−8.04(−14.80, −1.29), 0.021[a]
	After intervention	23.61 ± 4.25	17.59 ± 6.34	8.47(3.29, 13.66), 0.002[c]
	MD(95%CI), *P* value[b]	4.21(1.75, 6.66), 0.002	−9.85(−15.43, −4.27), 0.002	
TAC (mmol/L)	Baseline	1.43 ± 0.27	1.33 ± 0.26	0.10(−0.06, 0.27), 0.221[a]
	After intervention	1.54 ± 0.26	1.32 ± 0.23	0.15(0.03, 0.27), 0.011[c]
	MD(95%CI), *P* value[b]	0.10(0.04, 0.17), 0.003	−0.006(−0.05, 0.04), 0.775	
MDA (nmol/mL)	Baseline	49.00 ± 17.52	44.00 ± 15.38	5.00(−5.55, 15.55), 0.344[a]
	After intervention	42.40 ± 18.75	47.40 ± 12.73	−5.76(−15.20, 3.66), 0.222[c]
	MD(95%CI), *P* value[b]	−6.60(−0.40, −12.79), 0.038	3.40(−0.41, 7.21), 0.078	
hs-CRP (mg/L)	Baseline	2.29 ± 2.21	1.78 ± 1.96	0.51(−0.82, 1.85), 0.441[a]
	After intervention	1.64 ± 1.94	2.62 ± 2.64	−1.39(−2.53, −0.24), 0.019[c]
	MD(95%CI), *P* value[b]	−0.65(−1.09, −0.21), 0.006	0.83(0.27, 1.40), 0.006	
FBS (mg/dL)	Baseline	161.70 ± 41.62	188.70 ± 89.03	−27.00(−71.49,17.49), 0.227[a]
	After intervention	143.80 ± 43.69	197.05 ± 82.27	−34.84(−60.15, −9.52), 0.009[c]
	MD(95%CI), *P* value[b]	−17.90(−28.77, −7.02), 0.003	8.35(−5.21, 21.91), 0.213	

SOD: superoxide dismutase; GPX: glutathione peroxidase; TAC: total antioxidant capacity; MDA: malondialdehyde; hs-CRP: High sensitivity C reactive protein. The results are described as mean ± standard deviation (SD). [a]MD(95%CI), *P* value is reported based on the analysis of independent sample *t*-test. [b]MD(95%CI), *P* value is reported based on the analysis of paired sample *t*-test. [c]MD(95%CI), *P* value is reported based on the analysis of covariance.

Our findings were in accord with the results of Roozbeh et al. whose supplementation of end stage renal disease patients with 140 mg silymarin thrice daily for three weeks led to a significant increase in RBC GPX levels; in contrast to our results, a reduction was also observed in MDA levels (Roozbeh et al. 2011). Soto et al. in an experimental study of alloxan-induced diabetic rats, showed that silymarin protects the pancreatic cells from alloxan damage due to an increase in the activity of SOD, GPX and CAT in the pancreas (Soto et al. 2003). The study further suggested that silymarin, by increasing the pancreatic, hepatic and blood GSH levels, can prevent lipid peroxidation and MDA production induced by alloxan (Soto et al. 1998; Al-Jassabi et al. 2011). Soto et al. demonstrated that silymarin protects diabetic nephropathy and renal tissue damage induced by alloxan; this may be attributed to an increase in the recovery of gene expression and levels of antioxidant enzymes including SOD, GPX and CAT (Soto et al. 2010). Our results were in agreement with these results. These experimental studies support the findings of our study, except for the results pertaining to MDA. In our study, there was a significant within-group change in MDA levels for the silymarin group; however, no significant between-group differences were noted by the end of study course after adjusting for covariates. It is likely that this disagreement was due to differences in study design and the study population and their disease duration.

Since silymarin is a powerful free radical scavenger, several studies have proposed beneficial effects as a result of its supplementation in diseases with a pathophysiology closely related to free radicals, such as diabetes (Soto et al. 1998; Russell 2004). Free radical damage induced by oxidant agents or hyperglycaemia 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 by activating signalling pathway protein kinase C (PKC), mitogen-activated protein kinase (MAPK), the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and by transforming growth factor-β1 (TGF-β1) and monocyte chemoattractant protein-1 (MCP-1) involved in inflammatory pathways. It is claimed that these factors lead to pancreatic damage and disturbed glucose homeostasis (Soto et al. 1998; Al-Jassabi et al. 2011; Murata et al. 1998; Henriksen et al. 2011). The mechanisms underlying the antioxidative effects and pancreatic protection of silymarin have been explained in several experimental studies. Based on animal studies, silymarin induces gene expression and the activity of antioxidant enzymes SOD and GPX by stimulating DNA-dependant RNA polymerase I, leading to enhanced protein synthesis and glutathione content in the cell. In return, high levels of glutathione supply the sulphydryl groups necessary for DNA expression and the restoration of antioxidant enzyme synthesis by pancreatic cells, which is a possible defence against free radicals damages (Sotoa et al. 2003). Moreover, a desired ratio of GSH/GSSG contributes to favorable glucose homeostasis in diabetes (Sotoa et al. 2010). These potential mechanisms suggest that silymarin can increase the levels and activity of antioxidant enzymes (Sotoa et al. 2010). It has been demonstrated that silymarin inhibits oxidative damages in hepatic cell membranes (Turgut et al. 2008), microsomes and mitochondria (Bosisio et al. 1992) through the prevention of lipid peroxidation (Al-Jassabi et al. 2011). Lipid peroxidation products such MDA have been found to be high in T2DM patients; this mechanism may explain the MDA lowering properties of silymarin.

In particular, our study showed that supplementation of T2DM patients with three 140 mg silymarin tablets per day for 45 days remarkably decreased hs-CRP levels. This outcome was in agreement with results of other studies that have indicated the anti-inflammatory properties of silymarin and its potential for inhibiting the production of pro-inflammatory cytokines such as IL-1β, IL-2 and INF-γ through the suppression of various inflammatory pathways (Manna et al. 1999; Gharagozlooa et al. 2010). Previously, the anti-inflammatory characteristics and probable underlying mechanisms of silymarin had been demonstrated in cell line studies (Kang et al. 2004; Mandegary et al. 2013), which may explain the changes of hs-CRP levels observed in our study. It has been claimed that silymarin can inhibit the formation of 5-lypoxiganase products including lukotrienes and also inhibit enzymatic peroxidation in the lipoxygenase and cyclooxygenase pathways involved in inflammation (Gupta et al. 1999; Alarcon et al. 1992). Kang et al. indicated that silymarin suppresses IL-1β and PGE2 production by inhibiting the NF-kB/Rel transcription factor activation in mouse macrophages and RAW 264.7 cells (Kang et al. 2004). This effect of silymarin correlates with the suppression of IkBα phosphorylation. Additionally, in human histiocytic lymphoma U-937 cells, silymarin has been found to inhibit the activation of MEK and JNK signalling pathways induced by TNF-α (Manna et al. 1999). NF-kB/Rel transcription factor, MEK and JNK signalling pathways contribute to the production of various inflammatory mediators (Kang et al. 2004). Taken together, silymarin likely affects the hs-CRP levels as an inflammatory mediator through these mechanisms.

There were some limitations in the present study. First, the CAT and other inflammatory parameters such as IL-1 and TNF-α levels were not measured. Another limitation was the study's relatively small sample size and short intervention period. It is suggested that further studies devoid of these limitations be conducted. However, the triple blinding performed for random allocation and using a robust statistical analysis empowered the results of our clinical trial.

Conclusions

In conclusion, our results showed that silymarin supplementation improved antioxidant indices and reduced the inflammatory biomarker hs-CRP with a slight reduction in MDA levels in type 2 diabetic patients. Further investigation with a larger sample size and longer intervention time are needed to confirm the beneficial effects of silymarin in the management of diabetes complications, inflammatory parameters, antioxidant indices and MDA in type 2 diabetic patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2014.12.010.

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