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Research paper

Extract of *Dorema aucheri* induces PPAR-γ for activating reactive oxygen species metabolism

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ABSTRACT

Objective: The aim of the present study is to assess the potential anti-diabetic effect of *Dorema aucheri* extract through the peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway.
Marerials & methods: In this double-blind, placebo-controlled, randomized clinical trial, 170 patients with type II diabetes were randomly assigned to one of three groups, to receive a daily oral dose of either 200 or 500 mg of *D. aucheri* extract, or a placebo capsule for a period of 45 days. Blood samples were obtained from subjects and metabolic profiles, expression of PPAR-γ and the activity of related pathways, were assessed prior to the intervention and then again at day 45.
Results: The extract of *D. aucheri* was shown to influence reactive oxygen species (ROS) metabolic pathways. Notably, a reduction in the production of ROS by *D. aucheri* through induction of PPAR-γ was found. After 45 days of treatment with the extract of *D. aucheri* a significant increase in PPAR-γ expression was seen, with the 500 mg group displaying a 1.15–2.46-fold increase as distinct from the control group and pre-treatment ($P < 0.01$). The extract also caused a significant increment of plasma superoxide dismutase activity (from 1314.82 ± 137.99 to 1362.26 ± 129.90 U/g protein in the 200 mg group, $P = 0.001$, and from 1313.38 ± 134.24 to 1444.51 ± 140.42 U/g protein in the 500 mg treatment group, $P < 0.0001$) and vascular catalase gene activity (from 82.86 ± 9.13 to 86.04 ± 8.98 kU/g protein in the 200 mg group, $P = 0.003$, and from 79.71 ± 8.91 to 90.32 ± 10.18 kU/g protein in the 500 mg treatment group, $P < 0.0001$), when compared to the placebo group and baseline values. Both *D. aucheri* extract groups also displayed statistically significant reductions in mean plasma glucose, total cholesterol, triglycerides and low density lipoprotein cholesterol levels.
Conclusion: These findings indicate that this extract of *D. aucheri* exerts its biological effects through PPAR-γ activation, and suggests that it may behave as PPAR-α/γ dual activator. *D. aucheri* with its rich sesquiterpene content may serve as an alternative or adjuvant treatment for patients with diabetes.

1. Introduction

The plant *Dorema aucheri* (Fig. 1) belongs to the Apieaceae family and is endemic to the cold areas of Iran, especially the heights of the Alvand and Zagros mountains. *D. aucheri* is used by the local population for its medicinal properties, and is known locally by the names Bilhar and Oshaq. Studies of extracts of the aerial parts of *D. aucheri* have shown the presence of a large group of terpenoids, mainly the sesquiterpene compounds α-eudesmol, δ-cadinene and β-caryophyllene, which exhibit potent antioxidant activities (Table 1) (Masoudi et al., 2006; Moghadas et al., 2012; Wollenweber et al., 1995; Yousefzadi et al., 2011).

This medicinal plant and its sesquiterpene-rich extract have been investigated for their potential role in the treatment of thyroid disorders (Azarneushan et al., 2010), their effect on tumour growth, including breast cancer (Afshoon et al., 2010), the normalization of liver enzymes after carbon tetrachloride induced liver damage, and as a protection against oxidative stress (Sadeghi et al., 2005). *D. aucheri* has also been shown to increase the number of monocytes in the blood, and to inhibit the thromboxane levels of platelets under conditions of stress (Mokhtari et al., 2007).

Research indicates that the pharmacological properties *of D. aucheri,* including its anti-inflammatory and anti-cancer actions

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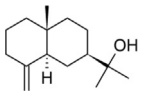
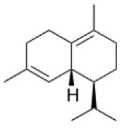
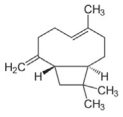
Fig. 1. *Dorema aucheri* plant (Alvand, Zagros mountains, Iran, April 2013).

Table 1
The main chemical constituents found in the aerial parts of D. aucheri, as extracted by hydrodistillation and analyzed by GC and GC/MS.

Compound name	Biochemical Structure	Composition%
α-eudesmol, a sesquiterpene		>31.2%
δ-Cadinen, a sesquiterpene		>10.9%
β-caryophyllene, a sesquiterpene		~4.9%

can be attributed to its sesquiterpene content (Guo et al., 2006; Kooa and Gang, 2012; Lin, 2012; Mokhtari et al., 2007; Pascual et al., 2001; Sadeghi et al., 2004). Sesquiterpenes from a variety of medicinal plants have been shown to influence different pathways, including the mitogen activated protein kinase (MAPK) signalling pathway, as well as exhibiting insulin-sensitizing activities (Lin, 2012; Wang et al., 2015; Zhang et al., 2009).

It has been suggested that the metabolism-regulating properties of sesquiterpene derivatives are mediated by activation of the peroxisome proliferator-activated receptor (PPAR) family, especially PPAR-γ. PPAR activation is in turn thought to reduce the atherogenic index, represented by total cholesterol/HDL ratio (Guo et al., 2006; Kooa and Gang, 2012; Lin, 2012; Pascual et al., 2001). PPAR-γ agonists also display a predominant role in the transactivation of superoxide dismutase (SOD) and catalase (CAT) genes (Gong et al., 2012; Okuno et al., 2008, 2010; Umeji et al., 2006; Yoo et al., 1999; Yukiko and Stanley, 2008). Importantly, SOD and CAT activity is positively associated with PPAR-γ activity in the aorta, both of which may be negatively related to aortic stiffness and lipid peroxidation (Umeji et al., 2006). The PPAR element is located in the sequence of the SOD and CAT genes, and participates in induction of these genes, thus improving peroxisome function by means of peroxisome proliferators, which contribute to the anti-atherosclerotic effects of PPARs (Okuno et al., 2008, 2010; Umeji et al., 2006; Yoo et al., 1999).

Sesquiterpenes found in traditionally used medicinal plants act as PPAR activators, and have, therefore, been proposed as potential treatments for diabetes, hepatitis, and hepatocellular carcinoma (Murphy and Holder 2000; Schlorff et al., 1999). Moreover, sesquiterpenes have been reported to behave as PPAR-α/γ dual agonists, a mechanism that furthers their therapeutic potential in the treatment of diabetes (Gong et al., 2012; Lin, 2012; Murphy and Holder 2000; Yoo et al., 1999; Zhao et al., 2012).

Research has shown that the PPAR-γ pathway controls the expression of genes involved in metabolic and oxidative pathways, and, therefore, has a major therapeutic role in treatment of diabetes and prevention of atherogenic activity and metabolic complications (Murphy and Holder 2000; Stephens et al., 2009; Vassort and Turan 2010). Some of the drugs are in current use for the treatment of type 2 diabetes, such as the thiazolidinediones, are PPAR-γ agonists.

The aim of the present study was to investigate the potential anti-diabetic effect of a sesquiterpene-rich extract of *D. aucheri* through the PPAR-γ pathway, evaluating its effect on expression of PPAR-γ in patients with hyperglycaemia and hyperlipidemia, as well as its effect on metabolic and oxidative biomarkers.

2. Materials and methods

2.1. Study population

One hundred and seventy male and female patients registered in Yazd Diabetes Research Center (affiliated to Shahid Sadoughi University of Medical Sciences, Yazd, Iran), with a positive diagnosis of type II diabetes, were enrolled into the study after signing a written consent form.

The sample size was determined by a biostatistician and was calculated using a variety of factors, including data obtained from previous studies (Rodriguez-Moran and Guerrero-Romero, 2003; Kudolo et al., 2006), the number of suitable patients available, the expected difference between the treatment and control groups with regards to serum concentration of cholesterol and glucose, and the anticipated distribution of data. In order to allocate subjects to treatment and control groups a randomisation sequence was generated using a random number table by a third party unaware of details of the experiment, with groups being matched for age and gender.

The inclusion criteria were a positive diagnosis of type 2 diabetes of at least one years' standing, being aged 40–65 years, no other major disease or drug consumption which might affect the variables of the study (at the discretion of the endocrinologist); no history of major illness during the past two months; no known allergy to *D. aucheri* and provision of written informed consent. Patients who were pregnant, breastfeeding, smokers, or receiving insulin therapy were excluded from the study. All participants were allowed to continue their oral antidiabetic drugs (as prescribed by their physician) throughout the study, but were

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asked not to make any alterations to their usual drug regimen. Incomplete compliance, lack of contact for more than two weeks, any change in routine medications, or intolerance to the extract would result in withdrawal from the study.

2.2. Study design

In this randomized, double-blind, placebo-controlled trial, the aerial parts (flowers, leaves and stems) of *D. aucheri* were collected from the mountains in the west of Iran (2300–3000 m above sea level), near the cities of Yasuj and Shahrekord, during the months of March and April. The plant was authenticated by Dr. Mosleh Arani, PhD in plant ecology from Yazd University, Iran, and a voucher specimen registered in the herbarium of Shahid Beheshti University, Tehran, Iran, under the code AR337E. These were dried, and an extract was obtained by hydrodistillation using a simple unit, according to the method outlined in previous studies (Masoudi et al., 2006; Moghadas et al., 2012; Wollenweber et al., 1995). Capsules were prepared by Barij Essence Pharmaceutical Co., Iran. The *D. aucheri* extract was encapsulated at doses of either 200 or 500 mg, under sterile conditions. No chemicals or excipients were present in the gelatine capsules other than the pre-weighed, powdered extract of *D. aucheri*. The placebo capsules were identical to the extract capsules in shape and colour and contained only 500 mg of wheat fibre.

The 170 participants were randomly divided into three groups. Group 1 (n = 58), the control group, received one placebo capsule per day, group 2 (n = 57) received 1 capsule containing 200 mg of *D. aucheri* extract per day, and group 3 (n = 55) received one capsule containing 500 mg of *D. aucheri* extract per day. The participants were instructed to take one capsule daily, one hour before lunch for 45 days, without making any adjustments to their diet and lifestyle. In order to ensure compliance and resolve any potential problems, each participant was contacted once a week during the study by one of the researchers.

The study protocol was approved by the Ethics Committee at Shahid Sadoughi University of Medical Sciences, with registration NO. 168931, and was conducted in accordance with The Declaration of Helsinki. The study is approved by the Iranian registry of clinical trials, with registration code IRCT2014123120502N1.

2.3. Biochemical analysis

2.3.1. Blood sampling

Blood samples were obtained from the subjects after a 12 h overnight fast at the beginning of study prior to intervention, and then again at the end of the trial at day 45. The analyses of antioxidant enzymes and PPAR expression were performed on the same blood samples.

2.3.2. Total RNA extraction

RNA was isolated from peripheral blood mononuclear cell (PBMC) samples using the Qiagen RNeasy Micro kit (Qiagen, Venlo, The Netherlands). RNA yield was quantified on a Nano-drop ND 1000 photometer (Nanodrop Technologies, Wilmington, USA), and RNA integrity was assessed after electrophoresis in non-denaturing 3% agarose gels by ethidium bromide staining (Vidal-Puig et al., 1997).

2.3.3. cDNA synthesis and quantitative real-time PCR (qPCR)

RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad Laboratories BV, Veenendaal, The Netherlands). Standard qPCR was performed with the use of Platinum Taq DNA Polymerase and SYBR Green on an iCycler PCR machine (Bio-Rad Laboratories BV, Veenendaal, The Netherlands) and repeated twice. The primers used in the PCRs were chosen based on the sequences available in PRIMERBANK (http://pga.mgh.harvard.edu/primerbank/index.html) (Vidal-Puig et al., 1997). qPCR data was normalized by measuring cycle threshold ratios between candidate genes and a housekeeping gene, human GAPDH, which was shown to be consistent within PBMCs. Data were expressed as ΔCt and $-\Delta\Delta$Ct normalized for the mean value of the plate (Bouwens et al., 2007; Vidal-Puig et al., 1997).

2.3.4. Activation assay of superoxide dismutase (SOD)

Total SOD activity was measured as described in Sarban et al. (2005) and Schlorff et al. (1999). One unit of SOD was defined as the amount of enzyme which inhibits oxidation of epinephrine by 50%, and expressed as units/mL of plasma/g protein.

2.3.5. Activation assay of CAT

Catalase (CAT) activity of plasma was measured according to the method described by Sarban et al. (2005) and Schlorff et al. (1999), with one unit of enzyme activity being equal to μM of H_2O_2 degraded/mL of plasma/g protein.

2.3.6. Trolox equivalent antioxidant capacity (TEAC) assay

The total antioxidant capacity of plasma and antioxidant gap were measured by employing the method of Miller and Rice-Evans (Savu et al., 2012), using a Trolox antioxidant standard curve.

2.3.7. Evaluation of metabolic profiles of plasma

All biochemical measurements were conducted using commercially available kits made by Pars Azmoon Co., Iran, according to the manufacturer's recommendations. The extent of protein glycosylation (HbA_{1C} %), the ratio of triglycerides (TG) to HDL-C concentration (TG/HDL-C) and total serum cholesterol (TC) to HDL-C concentration (TC/HDL-C), as important metabolic and atherogenic indices, were measured (Gurrola-Diaz et al., 2010; Neri et al., 2010).

2.4. Statistical analysis

A Student paired *t*-test was used to determine significant differences in metabolic status and qPCR gene expression between pre- and post-treatment values, and one-way ANOVA was used for comparisons of groups at the baseline and after treatment. Statistical significance for all experiments was accepted at $P < 0.05$.

3. Results

3.1. Participant demographics

Of 170 enrolled participants, 150 completed the 45-day intervention. Twenty participants were withdrawn from the study after randomization (8 from the control group, 7 from the 200 mg group, and 5 from the 500 mg group) for reasons unrelated to compliance with the study protocol (travel, surgery or death). All participants were accounted for, and Fig. 2 provides a schematic representation of the flow of participants through the course of the study. The remaining 150 participants were followed and all completed the 45 day treatment without any reported problems. The main clinical and laboratory characteristics of the participants are presented in Table 2, from which it can be seen that there were no statistically significant differences in demographic or biochemical characteristics between the three groups at baseline. (Fig. 2, Table 2).

3.2. D. aucheri extract acts as PPAR-γ activator

In this study, the extract of *D. aucheri* showed a considerable activation of PPAR-γ gene expression in patients with diabetes

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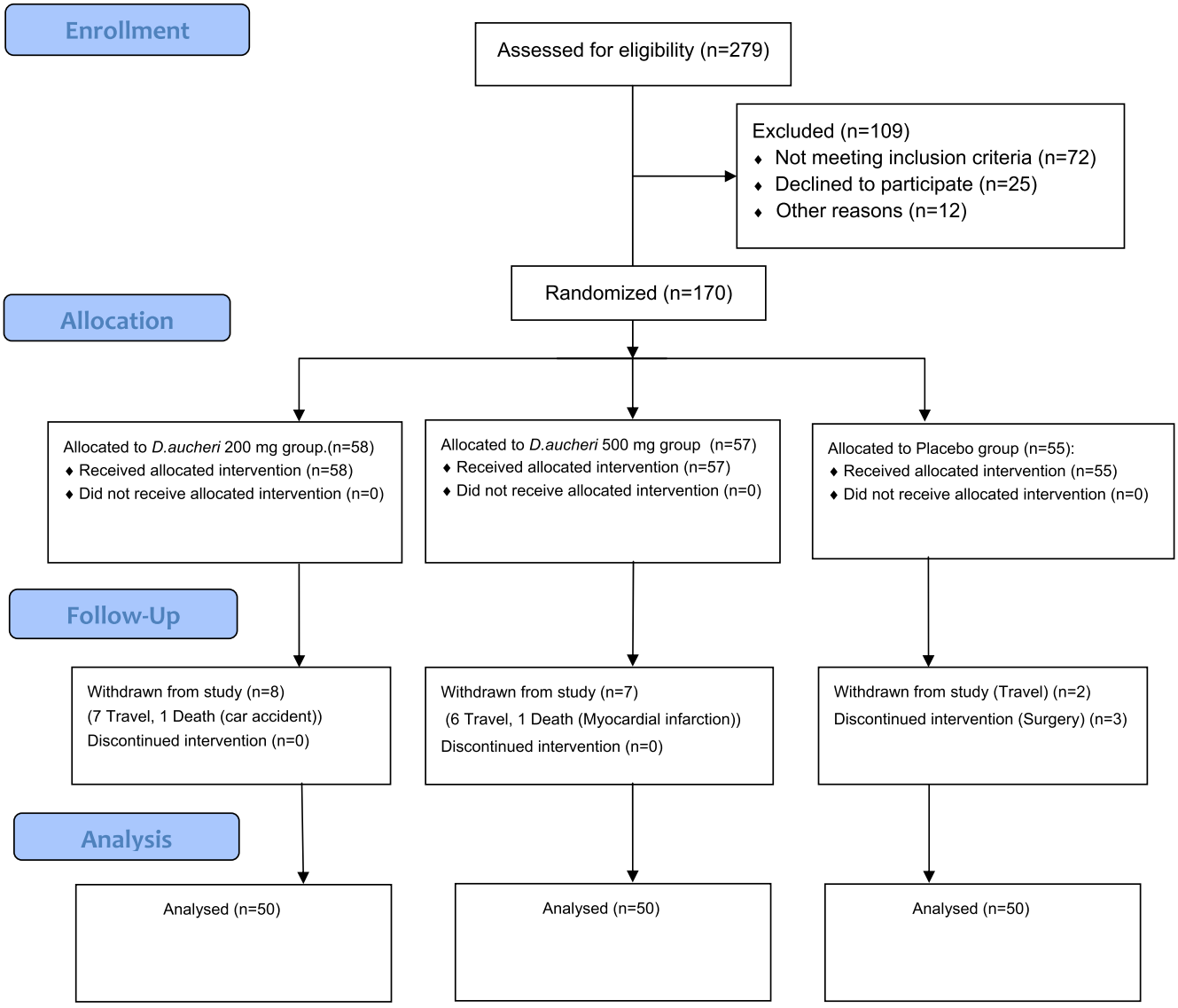

Fig. 2. Study Flow chart (CONSORT format).

(Fig. 3). Analysis of qPCR products from total RNA extracted from PBMCs, determined by the $2^{-\Delta\Delta Ct}$ formula, exhibited a 1.15-2.46 fold increase in induction of PPAR-γ gene with the 500 mg dose, when compared to the control group (with $2^{-\Delta\Delta Ct} = 1$, $-\Delta\Delta Ct \sim 0$). The average ratio of PPAR-γ expression in the 500 mg group was 1.6 ± 0.53 ($-\Delta\Delta Ct$ value). The 500 mg group was selected for the testing of PPAR-γ gene expression as it demonstrated the more significant metabolic response.

3.3. D. aucheri extract induces vascular SOD activity via PPAR-γ activation

Previous studies have shown that incubation of PBMCs with PPAR activators results in up-regulation of genes which play roles in metabolic pathways, primarily in β-oxidation (Bouwens et al., 2007; Vidal-Puig et al., 1997). The receptors recognize a tandem repeat of the AGGTCA motif located in the promoter of controlled genes such as SOD and CAT (Inoue et al., 2001; Yoo et al., 1999). When the PPRE are activated by PPARs, the SOD activity would increase, while the expression of the NF-κB p65 subunit and the production of pro-inflammatory cytokines, such as TNF-α would be decreased (Gong et al., 2012; Okuno et al., 2008, 2010; Yukiko and Stanley, 2008). Furthermore, activators of PPAR-γ gene expression would be activators of PPAR-α mRNA levels (Umeji et al., 2006; Yoo et al., 1999).

In light of the above, the induction and activation of SOD by the extract of *D. aucheri* was investigated as part of the present study. At a dose of 200 mg, the extract caused an average increment of SOD activity from 1314.82 ± 137.99 to 1362.26 ± 129.90 U/g protein after the 45 days of treatment ($P = 0.001$) and a more considerable average induction from 1313.38 ± 134.24 to 1444.51 ± 140.42 U/g protein was observed in the 500 mg treatment group ($P < 0.0001$), whereas there was no statistically significant alteration in average SOD activity in the placebo group, which was 1343.29 ± 144.44 U/g protein at baseline and 1331.11 ± 132.12 U/g protein at day 45 ($P = 0.447$) (Table 4).

3.4. D. aucheri extract induces vascular CAT activity via PPAR-γ activation

An enhancer region containing two functional PPAR-γ binding sites has been identified in the CAT gene (Okuno et al., 2008; Zhang et al., 2009). At the 200 mg dose, the extract caused a small increase in average CAT activity from 82.86 ± 9.13 to 86.04 ± 8.98

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Table 2
Demographic and biochemical characteristics of the study population with type II diabetes.

Parameters	Control (n = 50)	200 mg (n = 50)	500 mg (n = 50)	P value
Age (years)	53.02 ± 7.22	52.26 ± 7.89	51.24 ± 7.31	0.492
Sex (M/F)	23/27	26/24	30/20	0.18
FBS (mg/dL)				
Before treatment	182.44 ± 49.34	200.42 ± 45.22	195.72 ± 38.82	0.236
After treatment	180.04 ± 38.37	176.40 ± 41.74*	159.86 ± 40.10**	0.122
P value	0.101	0.019	<0.0001	
TC (mg/dL)				
Before treatment	261.16 ± 31.97	262.30 ± 33.81	264.82 ± 34.84	0.927
After treatment	259.64 ± 37.66	235.90 ± 23.29*	217.86 ± 26.39**	0.007
P value	0.110	0.024	< 0.0001	
TG (mg/dL)				
Before treatment	196.02 ± 33.16	194.74 ± 30.68	197.36 ± 32.00	0.753
After treatment	190.02 ± 41.77	173.00 ± 27.38*	160.68 ± 27.29**	<0.0001
P value	0.203	0.017	<0.0001	
LDL-C (mg/dL)				
Before treatment	147.9 ± 25.6	148.9 ± 25.5	148.6 ± 24	0.914
After treatment	154.1 ± 24.5	128.1 ± 23.4*	96.1 ± 36.2**	0.001
P value	0.321	0.010	<0.0001	
HDL-C (mg/dL)				
Before treatment	42.4 ± 5.4	41.9 ± 5.1	40.2 ± 5.7	0.852
After treatment	43.2 ± 8.1	46.7 ± 6.7*	50.4 ± 1.25**	0.034
P value	0.181	0.035	0.010	

FBS: fasting blood sugar; TC: total cholesterol; TG: triglycerides; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol.
Values are mean ± SD of 50 patients. *$P < 0.05$ vs. control group and pre-treatment values; **$P < 0.01$ vs. 200 mg group and control group and pre-treatment values. For a comparison of pre- and post-treatment values in the respective groups we used paired *t*-test, while differences between groups were analyzed by one-way ANOVA. Any *P*-value <0.05 was considered statistically significant.

kU/g protein after 45 days of treatment ($P = 0.003$). A more considerable average induction from 79.71 ± 8.91 to 90.32 ± 10.18 kU/g protein was observed with the 500 mg treatment dose ($P < 0.0001$). There was, however, no statistically significant change in CAT activity seen in the placebo group, which had an average of 80.78 ± 9.41 kU/g protein at baseline and 81.21 ± 8.76 kU/g protein at day 45 (Table 4). Fig. 3 shows that PPAR-γ mRNA expression was weak in patients receiving placebo in comparison with those treated with 500 mg of the extract.

3.5. D. aucheri extract regulates metabolic status

The glucose concentration (FBS), total serum cholesterol (TC), triglyceride (TG), and LDL-C levels were high across all three

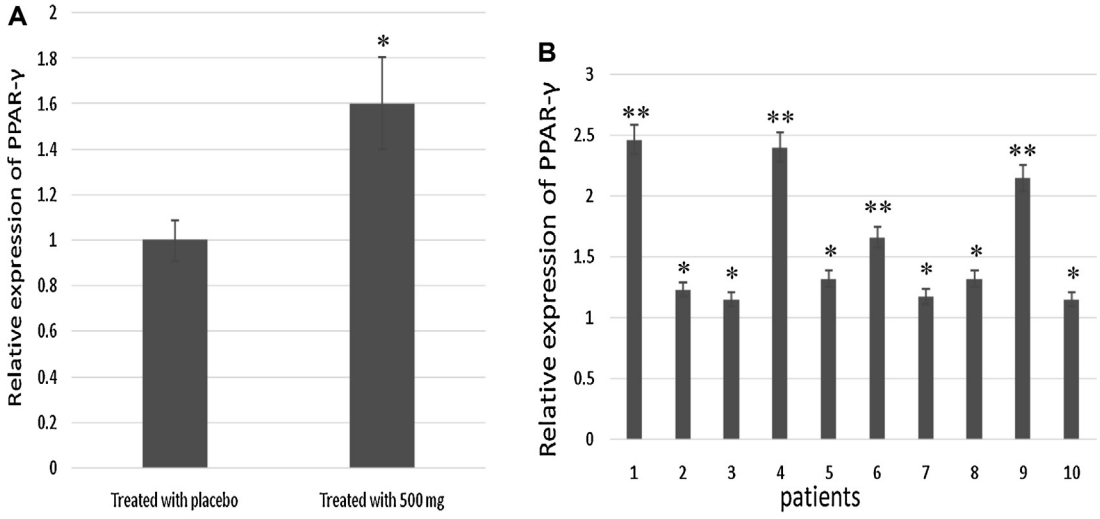

Fig. 3. PPAR-γ mRNA expression levels after 45 days of treatment with *D. aucheri* extract or placebo (A) Real-time PCR analysis of PPAR-γ mRNA relative expression levels ($2^{-\Delta\Delta Ct}$) in the placebo and 500 mg *D. aucheri* groups. (B) Changes in PPAR-γ mRNA expression levels in the 10 patients in the 500 mg extract group who expressed the highest positive metabolic response. GAPDH mRNA expression levels were performed as control. Values are mean±SD of 10 patients, in pre- and post-treatment phases. *$P < 0.01$ vs. control group and pre-treatment; **$P < 0.001$ vs. control group and pre-treatment. Student *t*-test was used for comparison of pre- and post-treatment values in the respective groups and between groups. *P*-values <0.05 were considered to be statistically significant.

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Table 3
The primer sequences used for real-time PCR.

Gene	Accession No.	Forward primer	Reverse primer
PPAR-γ	NM_005037.5	5′-TCAAGTTCAAACACATCACCCC-3′	5′-AGGAGAGTTACTTGGTCGTTCA-3′
GAPDH	NM_001256799.2	5′-GTGACTAACCCTGCGCTCC-3′	5′-AGTTAAAAGCAGCCCTGGTGA-3′

groups at baseline, with no significant difference between groups ($P = 0.236$-0.927) (Table 2). After the 45-day treatment with *D. aucheri*, FBS, TC, TG, LDL-C showed significant regulation, which was most markedly demonstrated ($P < 0.0001$ to 0.010) by the 500 mg dosage group which showed a 31.2% reduction in the average FBS level from 195.72 ± 38.83 to 159.86 ± 40.10 mg/dL, a 23.7% reduction in average TC from 264.82 ± 34.84 to 217.86 ± 26.39 mg/dL, a 25.6% reduction in TG from 197.36 ± 32.00 to 160.68 ± 27.29, and a 35.3% reduction in average LDL-C levels from 148.6 ± 24 to 96.1 ± 36.2 mg/dL. The 500 mg dose group also showed a 23.2% increase in average HDL-C levels from 40.2 ± 5.7 to 50.4 ± 1.25 mg/dL ($P = 0.010$) (Table 3). The 200 mg dose group also displayed a 12% reduction in average FBS levels, from 200.42 ± 45.22 to 176.40 ± 41.74 mg/dL, as well as a 10% reduction in average TC, from 262.30 ± 33.81 to 235.90 ± 23.29 mg/dL, an 11% reduction in average TG, from 194.74 ± 30.68 to 173.00 ± 27.38 mg/dL, and a 14% reduction in LDL-C, from 148.9 ± 25.5 to 128.1 ± 23.4 mg/dL, as well as an 11% increase in average HDL-C, from 41.9 ± 5.1 to 46.7 ± 6.7 mg/dL, all of which were statistically significant ($P = 0.10$ to 0.035).

3.6. D. aucheri extract decreases the atherogenic index (TC/HDL-C) and insulin resistance status (TG/HDL-C) via PPAR-γ activation

In contrast to the induction of SOD and CAT activity by PPARs, the atherogenic index (TC/HDL-C) and insulin resistance status (TG/HDL-C) were conversely regulated by the extract. Both indices are useful tests to detect insulin resistance and risk of cardiovascular disease (Gurrola-Diaz et al., 2010). As can be seen from Fig. 4 and Table 2, both indices were significantly decreased by treatment with 200 mg and 500 mg doses of *D. aucheri*, when compared to the control group and baseline values ($P < 0.01$).

3.7. Effect of the D. aucheri extract on vascular oxidative status

Both the *D. aucheri* treatment groups had significantly higher levels of TEAC than the placebo group after 45 days of treatment. As Table 4 shows, the 200 mg and 500 mg groups displayed a 6% and 11% rise in average levels of TEACs respectively. This was a statistically significant increase in comparison to the control group ($P < 0.01$).

4. Discussion

D. aucheri is an Iranian medicinal plant with known traditional use in the treatment of metabolic disorders, its molecular mechanism(s) of action are not, however, fully understood. PPARs are members of the nuclear receptor superfamily. Their agonists are currently prescribed as anti-hyperlipidemic and anti-hyperglycemic drugs. Naturally derived sesquiterpenes have been shown to enhance PPAR-γ activation by directly binding to the PPAR-γ ligand binding domain. Additionally, sesquiterpenes stimulate the transactivation of PPAR-dependent gene promoters. Previous works strongly indicate that sesquiterpenes are active phytochemical constituents that exert an anti-diabetic effect through the PPAR-γ pathway. Moreover, sesquiterpenes behave as PPAR-α/γ dual activators, increasing their therapeutic potential for the treatment for diabetes (Lin, 2012).

Sesquiterpenes from medicinal plants have been shown to exert their metabolic regulatory activities through different mechanisms involving ROS scavenging activity and the MAPK signalling pathway (Lin, 2012; Wang et al., 2015; Zhao et al., 2012, 2015).

Sesquiterpenes have also shown strong insulin-sensitizing activities. This potential anti-diabetic effect of sesquiterpenes has been found to be through the insulin pathway, possibly through the inhibition of protein tyrosine phosphatases, especially PTP1B, thus promoting glucose uptake by GLUT4 (Zhang et al., 2009).

Inflammation and oxidative stress have been implicated as relevant factors in the pathogenesis of atherosclerosis and the macrovascular and microvascular complications of diabetes, as well as in beta cell failure in insulin resistance (Okuno et al., 2008, 2010; Zhao et al., 2015). Oxidative stress can induce expression of chemokines and the protein levels of IKKβ/p-IKKβ, IκBα and NF-κB/p-NF-κB in monocytes (Zhao et al., 2015). Herbally derived sesquiterpeneshave also been shown to exhibit anti-inflammatory effects (Lin, 2012; Wang et al., 2015; Okuno et al., 2008; Zhao et al., 2012, 2015). These findings provide a novel explanation for the anti-diabetic effect of sesquiterpenes, and suggest them to be of potential benefit against the vascular complications associated with diabetes and as an alternative or adjuvant to insulin therapy (Lin, 2012; Wang et al., 2015; Zhang et al., 2009; Zhao et al., 2012, 2015).

Table 4
Anti-oxidant status of the study population before and after treatment with *D. aucheri* extract or placebo.

Parameters	Control (n = 50) mean ± SD	200 mg (n = 50) mean ± SD	500 mg (n = 50) mean ± SD	*P value*
SOD (U/g protein)				
Before treatment	1343.29 ± 144.44	1314.82 ± 137.99	1313.38 ± 134.24	0.521
After treatment	1331.11 ± 132.12	1362.26 ± 129.90*	1444.51 ± 140.42**	0.003
P value	0.447	0.001	<0.0001	
CAT (kU/g protein)				
Before treatment	80.78 ± 9.41	82.86 ± 9.13	79.71 ± 8.91	0.221
After treatment	81.21 ± 8.76	86.04 ± 8.98*	90.32 ± 10.18*	0.028
P value	0.462	0.003	<0.0001	
TAC (mM)				
Before treatment	0.57 ± 0.03	0.58 ± 0.031	0.59 ± 0.025	0.416
After treatment	0.56 ± 0.02	0.62 ± 0.032*	0.67 ± 0.019**	0.039
P value	0.38	0.041	0.006	

SOD: vascular superoxide dismutase; CAT: catalase; TAC: total antioxidant capacity as measured by Trolox equivalent antioxidant capacity.

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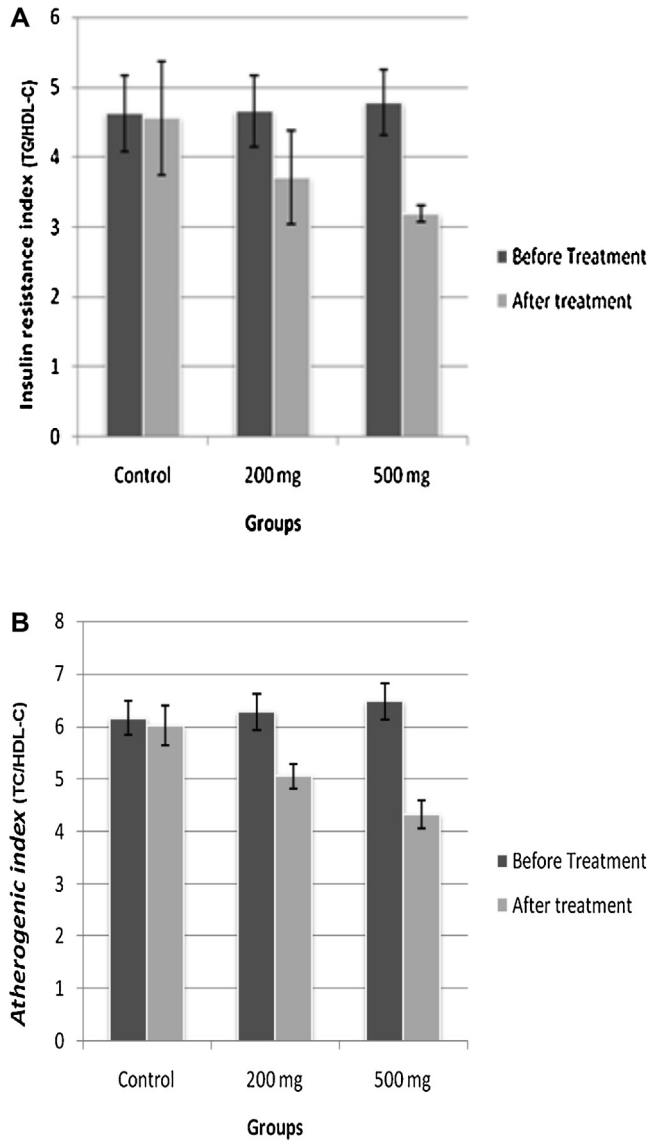

Fig. 4. Atherogenic index (TC/HDL-C) and insulin resistance status (TG/HDL-C) of subjects with type II diabetes at baseline and after treatment with *D. aucheri* or placebo. Values are mean ± SD of subjects in each group, in pre- and post-treatment phases. $^{*}P < 0.01$ vs. control group and pre-treatment values; $^{**}P < 0.01$ vs. 200 mg and control group and pre-treatment values. Student paired *t*-test was used for comparison of pre- and post-treatment values in the respective groups, while differences between groups were compared by one-way ANOVA, *P*-values < 0.05 were considered to be statistically significant. TC: Total cholesterol; TG: triglycerides; HDL-C: HDL- cholesterol.

Herbal sesquiterpenes are able to stimulate transactivation of both PPAR-α/γ-dependent gene promoters. Results of previous studies indicate that PPAR-γ agonists exert an anti-diabetic effect through both PPAR-α/γ pathways. Moreover, sesquiterpenes are believed to behave as PPAR-α/γ dual agonists so they are proposed as a useful potential treatment for diabetes (Lin, 2012; Umeji et al., 2006; Yoo et al., 1999; Gong et al., 2012; Zhao et al., 2012; Murphy and Holder, 2000). PPAR-γ activators, in particular, have demonstrated various effects, including adipocyte differentiation, lipid metabolism, anti-inflammatory, anti-diabetic, and anti-atherosclerotic activity.

The effects of PPAR activators and peroxisome proliferators are dose-dependent (Lin, 2012; Yukiko and Stanley, 2008; Umeji et al., 2006; Zhao et al., 2012), a fact that is supported by the present study, which found that many of the results in both *D. aucheri* treatment groups were statistically significant when compared to control and baseline values, but often displayed a more pronounced effect in the 500 mg than 200 mg group.

PPAR-α/γ are expressed ubiquitously in the vascular system as well as in adipose tissues and PBMCs (Yukiko and Stanley, 2008; Vidal-Puig et al., 1997; Bouwens et al., 2007). According to Murphy and Holder (2000) PPAR-γ is expressed ubiquitously in all tissues of adult mammals. SOD, CAT and PPAR-γ play crucial roles in the anti-atherogenic effects of anti-hyperlipidemic drugs and in hypercholesterolemia *in vivo* (Zhang et al., 2009; Okuno et al., 2008, 2010; Yukiko and Stanley, 2008; Umeji et al., 2006; Yoo et al., 1999; Gong et al., 2012). Research also indicates that herbally derived sesquiterpene activators could enhance PPAR-γ activity and also induce its interaction with PPRE (PPAR-γ response element), SHP, and ABCA1 gene promoters, in a dose-dependent manner (Lin, 2012; Zhao et al., 2012).

The chemical composition of other researched traditional herbal medicines with PPAR-α/γ agonistic activity is similar to *D. aucheri* in their containing sesquiterpenes (Pascual et al., 2001; Kooa and Gang, 2012; Lin, 2012; Zhao et al., 2012). Extracts of ginger and turmeric, for example, contain a large variety of terpenoids, including the sesquiterpene eudesmols and cadinenes, which possess anti-diabetic and anti-oxidant properties. Both ginger and turmeric sesquiterpenes exhibit hypoglycaemic effects in type 2 diabetic mice via PPAR-γ activation and there is a synergistic effect when both these sesquiterpenoids are applied together (Pascual et al., 2001; Kooa and Gang, 2012; Guo et al., 2006; Lin, 2012; Zhao et al., 2012). Herbal sesquiterpenes have also exhibited the ability to modulate ROS formation in different cell types (Pascual et al., 2001; Kooa and Gang, 2012; Wang et al., 2015; Zhang et al., 2009).

The present study demonstrates an extract of *D. aucheri* to both significantly control the atherogenic index, and regulate metabolic status. The *D. aucheri* extract has also been shown to effectively elevate the HDL/LDL ratio and lower triglyceride levels in hyperlipidemic patients, possibly through the regulation of a series of genes involved in lipid or sugar metabolism. These results could, at least in part, be explained by the observed anti-hyperlipidemic and anti-hyperglycemic effects of *D. aucheri* in humans through the PPAR-α/γ pathway and its inhibitory effects on oxidative stress via the activity of SOD and CAT and through PPAR-γ regulation.

PPAR-γ agonists not only improve insulin sensitivity but also reduce oxidative stress. In mice, SOD and CAT, as major anti-oxidant enzymes are directly regulated by PPAR-γ through two PPAR-γ binding elements in its promoter (Okuno et al., 2008, 2010; Yukiko and Stanley, 2008; Umeji et al., 2006; Yoo et al., 1999; Gong et al., 2012). This study also confirmed the regulatory mechanisms of SOD and CAT activity by PPAR-γ expression in humans. In addition, PPAR-γ agonists suppress the major sources of oxidants within the vascular system, including phagocytic NADPH oxidase and endothelial NADPH oxidase (Guo et al., 2006; Yukiko and Stanley, 2008; Umeji et al., 2006; Murphy and Holder, 2000).

Several studies have shown the beneficial effects of over-activation and/or induction of SOD and CAT in the vascular system, which include decreasing superoxide levels in endothelial cells, suppression of age-related oxidative stress, protection against inflammatory events by inhibiting NF-κB p50/p65 heterodimer activation, and suppression of LDL oxidation (Okuno et al., 2008, 2010; Yukiko and Stanley, 2008; Umeji et al., 2006; Yoo et al., 1999; Gong et al., 2012; Zhao et al., 2012; Murphy and Holder, 2000; Stephens et al., 2009; Vassort and Turan, 2010). Substantial ROS production has been found to activate MAPK signalling cascades (Pascual et al., 2001; Kooa and Gang, 2012; Wang et al., 2015; Zhang et al., 2009). Suppression of ROS production has been found to cause a significant reduction in the phosphorylation of p38 and JNK

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MAPKs, without altering the phosphorylation level of ERK1/2 MAPK in pancreatic β-cells (Wang et al., 2015).

Importantly, MAPKs that mediate ROS-induced pancreatic β-cell apoptosis are reported to be inhibited by sesquiterpenes isolated from medicinal plants, which regulate the insulin pathway and are able to protect different cell types against ROS (Pascual et al., 2001; Kooa and Gang, 2012; Lin, 2012; Wang et al., 2015; Zhang et al., 2009; Murphy and Holder, 2000). Taken together, these reports confirm that herbally derived sesquiterpenes are able to improve cell survival by impairing the activation of p38 and JNK MAPKs in high glucose and ROS stimuli (Pascual et al., 2001; Kooa and Gang, 2012; Lin, 2012; Wang et al., 2015; Zhang et al., 2009; Zhao et al., 2015). Studies show that inhibition of p38 and JNK significantly block the apoptotic response in high glucose-treated cells, which is associated with ROS generation. Moreover, sesquiterpenes extracted from herbs have shown the ability to protect cells from apoptosis through inhibition of ROS generation (Lin, 2012; Wang et al., 2015; Zhao et al., 2012, 2015).

The effects outlined above are in agreement with the findings of the present study, which confirms the links between an extract of a sesquiterpene rich herb, anti-oxidant enzyme activation, cell survival pathways, as well as PPAR-γ induction (Sadeghi et al., 2004; Pascual et al., 2001; Kooa and Gang, 2012; Guo et al., 2006).

The therapeutic benefits of sesquiterpene anti-oxidants in diabetes and atherosclerosis might be caused by alterations in the molecular regulation of gene expression in vasculature, muscle, and inflammatory cells including the suppression of ROS-induced gene expression in vascular cells (Yukiko and Stanley, 2008; Vidal-Puig et al., 1997; Bouwens et al., 2007; Yoo et al., 1999; Zhao et al., 2015). The present study found significant increases in PPAR-γ expression and in the activity of anti-oxidant enzymes SOD and CAT after a 45-day treatment with *D. aucheri*. Accordingly, sesquiterpene rich herbal treatments would increase PPAR-γ DNA binding activity. Our findings suggest that sesquiterpenes from *D. aucheri* are PPAR-γ activators that activate PPARs to transactivate target genes in a concentration-dependent manner.

In data collected during the present study, but as yet unpublished, it was observed that the extract of *D. aucheri* could also significantly reduce advanced oxidation protein products (AOPPs) and oxidative biomarkers of lipid peroxidation (i.e., malondialdehyde), carbonyl groups and advanced glycosylation end products (AGEs) in diabetic patients (unpublished results).

This study showed a modest increase in the mRNA expression and activity levels of PPAR-γ and anti-oxidant enzymes SOD and CAT at a dose of 500 mg/day of *D. aucheri* extract. Although it has been suggested that over-activation of PPAR-γ signalling may contribute to the development of insulin resistance (Murphy and Holder, 2000), this paradoxical phenomenon was not observed in the present study but might have been observed, given the outlined dose-dependent action of PPAR-γ activators, had a broader dosage range of extract been tested.

The results of the present study are suggestive of a therapeutic potential for *D. aucheri,* in the treatment of patients with type 2 diabetes and merit further clinical investigation. A clinical trial, for example, comparing the activity of *D. aucheri* with standard PPAR-γ agonist drug treatment would be useful.

Conflict of interests

The authors declare that there is no conflict of interest.

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