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Role of resveratrol supplementation in regulation of glucose hemostasis, inflammation and oxidative stress in patients with diabetes mellitus type 2: A randomized, placebo-controlled trial

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ABSTRACT

Objective: The objective was to determine the effects of resveratrol supplementation on glucose homeostasis, oxidative stress, inflammation and microRNAs expression in patients with diabetes mellitus type 2 on oral hypoglycemic drugs.

Method: This was a randomized, double blinded placebo-controlled parallel group trial. The diabetic patients (n = 110) were randomly assigned either to resveratrol (n = 55) and placebo (55) groups after informed consent and given once daily resveratrol 200 mg and cellulose capsules respectively for 24 weeks. Fasting glucose, insulin, HbA1c, lipid profile, TNF- α, IL-6, hs-CRP, MDA & circulatory microRNAs were measured at start and end of 24- week intervention.

Results: Out of 110 patients recruited, 94 patients completed the study comprising of 45 in resveratrol and 46 in placebo group. The resveratrol supplementation after 24 weeks was resulted in significant reduction [mean difference (95%CI)] of plasma glucose[− 0.50(−0.94 to −0.06)], insulin[− 1.31(−2.24 to −0.38)], homeostatic model assessment of insulin resistance[− 0.83(−1.37 to −0.29)], malondialdehyde[− 0.36(−0.61 to −0.11)], high sensitive-C-reactive protein[− 0.35(−0.70 to −0.01)], tumor necrosis factor-alpha[− 1.25(−1.90 to −0.61)] and interleukin-6[− 1.99(−3.29 to −0.69)]. More than two-fold down regulation in miRNA-34a, miRNA-375, miRNA-21, miRNA-192 and up regulation in miRNA-126 and miRNA-132 expression was noted in patients receiving resveratrol as compared to placebo. No side effects were reported during the trial.

Conclusion: Resveratrol supplementation contributes in improvement of glycemic control by reducing insulin resistance. It has significant beneficial impact on chronic inflammation, oxidative stress and associated microRNA expression in diabetic patients. Thus, supplementation of resveratrol along with oral hypoglycemic agents may be useful in the reduction of diabetic associated complications.

1. Introduction

Type 2 Diabetes mellitus (T2DM) is a chronic hyperglycemic state either due to ineffective production of insulin from pancreatic beta cells or the presence of increased insulin resistance in the body. Continuous modifications in human's lifestyle including unhealthy diet pattern, physical inactivity and increase occurrence of obesity have brought a global increment in the prevalence of DM. In 2017 it was estimated that 425 million people worldwide are suffering from T2DM and it is expected to increase up to 629 million by the year 2045. [1]

Uncontrolled hyperglycemia has a significant association with persistent oxidative stress and chronic low-grade inflammation. Association of oxidative stress and inflammation with development of insulin resistance is considered a main pathogenic mechanism for the persistent increase in the fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c) and homeostatic model assessment of insulin resistance (HOMA-IR) in patients of T2DM. [2] Regarding oxidative stress, malondialdehyde (MDA) is one of the most frequently used biomarkers that provide a better indication of the overall lipid peroxidation status in the plasma. [3] Moreover tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) act as effective inflammatory indicators.[2,4].

Currently, circulatory micro-ribonucleic acids (miRNAs) are studied to have a role in the regulation of glucose metabolism, insulin resistance,

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monitoring of inflammation and oxidative stress. These properties suggest them as the novel, best therapeutic biochemical markers for diagnosis and treatment of T2DM. [5]

Uncontrolled diabetes is a potential threat to induce various complications in the body. To prevent and treat T2DM and its life-threatening co-morbidities, the use of appropriate nutritional supplement along with recommended diabetic therapy has risen to prominence. [6] To maintain glucose homeostasis, oral hypoglycemic agents (OHGA) are routinely used in the T2DM. [7] We make a hypothesis that in patients with T2DM, a natural dietary supplement which has anti-oxidant and anti- inflammatory property may have additive effect for improvement of glycemic status and decrease the progression of disease at a molecular level along with already prescribed OHGA.

Resveratrol is non-flavonoid stilbene derivative polyphenol. Structurally, it is a 3,5,4′ – trihydroxystilbene. Dietary source of resveratrol include grapes, peanuts, cocoa and a large range of fruits including bilberry, cranberry, blueberry. [8] A meta-analysis revealed that resveratrol dose $\geq$ 100 mg/day presented more favourable results. [9] The dose of resveratrol up to 1 g is considered to be generally safe in human beings. [8] Resveratrol exhibits effective glucose control, anti-inflammatory and anti-oxidative properties in various animal models.[6,10]

In human beings, a few resveratrol studies have been conducted to find out the effectiveness of resveratrol on glycemic control and associated biochemical markers in T2DM. The limitations of the studies were the small number of patients and the short duration of clinical trials. [11] Therefore further studies are still required to prove the efficacy of resveratrol in T2DM. [12] As the data is very limited and the effectiveness of resveratrol in patients with T2DM is controversial, we planned this randomized clinical trial (RCT) to explore the effectiveness of resveratrol in diabetic patients.

This RCT was carried out to find out the effect of Resveratrol supplementation (200 mg/day) along with prescribed OHGA on glucose homeostasis (FPG, insulin, HbA1c, HOMA-IR), inflammation (TNF-α, IL-6, hs-CRP), oxidative stress (MDA), and miRNAs (miRNA-21, miRNA-34a, miRNA-126, miRNA-132, miRNA-192 and miRNA-375) expression in patients with T2DM.

2. Materials and methods

2.1. Study design and setting

This was a randomized, double-blind, placebo-controlled parallel-group trial with a 1:1 allocation ratio. The study was carried out at the Armed Forces Institute of Pathology, NUMS in collaboration with Mega Medical Complex Hospital, Rawalpindi, Pakistan. The duration of the study was 15 months starting from July 2018 up to September 2019. The study protocol was approved by the Ethics Review Committee of Armed Forces Institute of Pathology (Approval no: PhD-Path-18–02/Read-IRB/247; Approval date: 5 April 2018), and was registered with the Sri Lankan Clinical Trial Registry (SLCTR/2018/019).

2.2. Patients

Both men and women aged 18–70 years, having a duration of diabetes $\geq$ 5 years, HbA1c 7–12% and on treatment with OHGA for at least 1 year were included in the study. Exclusion criteria comprised of the history of acute disease, thyroid illnesses, malignant disorders, HIV, hepatitis B or hepatitis C, uncontrolled hypertension and chronic kidney disease. Patients having a body mass index (BMI) more than 35 kg/m2, familial hyperlipidemic disorder, pregnancy or lactation or history of using insulin, statins, anti-inflammatory drugs, or vitamin supplements were also excluded from the study. Patients were enrolled in the study after obtaining written informed consent. Clinical history and data comprising of age, gender, weight, height, disease history, results of the previous laboratory tests and continued treatments were collected and recorded.

2.3. Randomization and intervention

A total of 304 T2DM patients were enrolled. After screening 29 patients were excluded; 18 did not meet inclusion criteria and 11 declined participation. A total of 275 patients met the eligibility criteria of the clinical trial. These patients were enrolled in the study and equally divided into the resveratrol (n = 55), tocotrienol (n = 55), vitamin D (n = 55), mixture (n = 55) and placebo (n = 55) groups through a simple random draw. Here we have presented the data of resveratrol (n = 55) and placebo (n = 55) groups (Fig. 1). Capsules, either resveratrol (200 mg) or placebo (cellulose) were identical in shape, size and colour. Mega Resveratrol was 99% Pure Trans-resveratrol (Candlewood Stars Inc, Danbury, USA). It was purchased in powder form and customized in capsule form in the laboratory. Allocation of treatment was a double-blind procedure for both participants and the researcher. The only pharmacist was aware of the details of capsules. The researcher randomly assigned capsules to participants. The participants were advised to take a capsule daily with breakfast for 24 weeks along with their already prescribed OHGA. The patients were taking OHGA including metformin, vildagliptin, glimepiride, gliclazide and glibenclamide. Patients were guided regarding the continuous use of OHGA and monitoring of blood glucose during the clinical trial. To ensure the patient's compliance and to find out any adverse effect, patients were monitored through phone calls at the interval of 2 weeks. Regular follow-up visits were planned at 6 weeks. In these visits, patients were asked about any unwanted effects of the drug. Liver functions tests were performed as required. Returned capsules were also counted to check compliance with treatment.

2.4. Laboratory biochemical analysis

Total 10 ml blood was collected for analysis of FPG, fasting insulin, HbA1c, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), triglycerides, low-density lipoprotein- cholesterol (LDL-C), TNF-α, IL-6, hs-CRP, MDA & miRNAs and 20 ml spot urine sample was taken for microalbuminuria both at commencement and at the end of the intervention. The laboratory analysis was done at the Armed Forces Institute of Pathology according to standard validated laboratory protocols.

FPG, HbA1c, total cholesterol, HDL-C, LDL-C, triglycerides, urinary albumin and urinary creatinine were analyzed on the automated chemistry analyzer, ADVIA Centaur (Siemens, Tarrytown, NY, USA). Serum hs-CRP was estimated using IMMULITE® 1000 (Siemens, Tarrytown, NY, USA). Serum insulin was measured on ADVIA Centaur® XP immunoassay (Siemens, Tarrytown, NY, USA). Serum TNF-α, IL-6, and MDA were measured by ELISA kits (Elabscience, Texas, USA). Microalbuminuria was detected through an albumin creatinine ratio (ACR). HOMA-IR was calculated by the formula: Insulin (μU/ml) x Glucose (mmol/l)/22.5.

2.5. Real-time quantitative reverse transcription PCR

Total RNA, including miRNA, was extracted from whole blood using trizol LS method. [13] miRNA was extracted within 1 h after taking the sample from patients. During the process of extraction, after the step of lysis, 3.5 μl of *Caenorhabditis* Elegans (cel) miRNA-39 was added in the sample as spike-in control; Cat No. 219610 (Applied Biological Materials Inc, Richmond, BC, Canada) to adjust sample variation. The purity and concentration of total RNA were assessed by NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). RNA samples were saved in RNA screw cap microtubes; Cat No. GTR5100-S (GenTegra, Pleasanton, CA, USA) for a maximum period of 2 months. cDNA was synthesized using miRNA cDNA Synthesis Kit, with Poly(A) Polymerase Tailing; Cat No. G903 (Applied Biological Materials Inc, Richmond, BC, Canada). Quantitative reverse transcription- PCR (qRT-PCR) was done on real-time PCR system 7500 (Applied Biosystems; Thermo Fisher Scientific Inc, Foster City, California, United

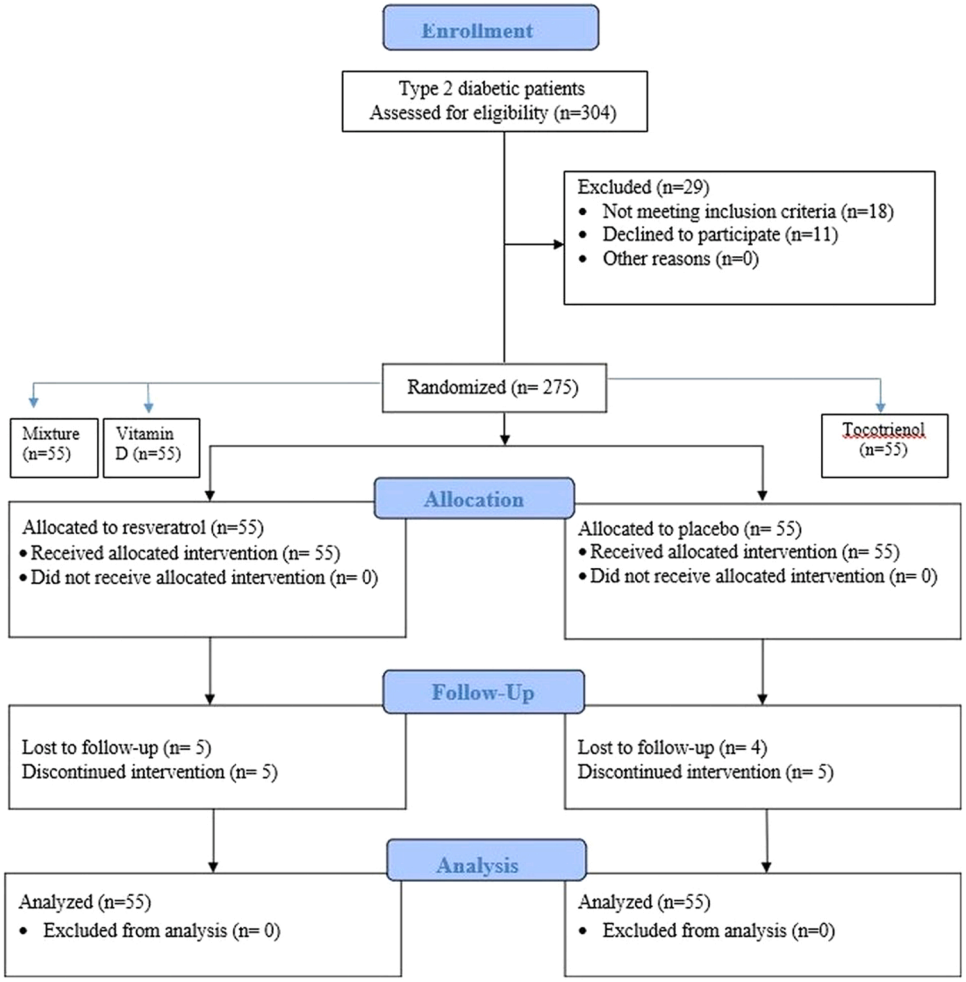

Fig. 1. CONSORT flow chart.

States) using Bright Green miRNA qPCR Master Mix-Low ROX (Applied Biological Materials Inc, Richmond, BC, Canada) and hsa-miRNA specific primers (Applied Biological Materials Inc, Richmond, BC, Canada). All samples were run in triplicate. Cel- miRNA-39–3p (Applied Biological Materials Inc, Richmond, BC, Canada) was used as an internal control reference gene for the normalization of data. Quantification cycles (Cq) values ≥ 35 were considered as negative amplification. ΔCq was used to represent the differences between the Cq value of miRNAs resveratrol/placebo group and Cq value of cel-miR-39 (ΔCq = mean Cq-miRNA (treatment group) – mean Cq-cel- miR-39). miRNA expression change was presented as expression fold change ($2^{-\Delta\Delta Cq}$) relative to baseline. Negative fold change due to treatment was calculated by formula = - 1/fold change ($2^{-\Delta\Delta Cq}$) and positive fold change were written down as such. [14]

2.6. Study outcomes

Primary outcome variables included were mean reduction in HbA1c, hsCRP and MDA after taking resveratrol supplementation in comparison with placebo at 24 weeks. Secondary outcome measures comprised of mean change in FPG, fasting insulin, HOMA-IR, lipid profile, microalbuminuria, TNF-α, IL-6 and miRNAs in the resveratrol group as compared with placebo at 24 weeks.

2.7. Sample size calculation

Calculation of sample size was done through G Power 3.1 software (Experimental Psychology institute, Dusseldorf, North Rhine-Westphalia, Germany) available online using 80% power and 95% confidence level. Based on previous studies, primary endpoint variables including post-treatment CRP effect size, mean change in HbA1c and MDA levels were considered. The highest calculated sample size was 44 individuals in each group (total 88 patients), based upon − 0.78 post-treatment CRP effect size in both male and female. [15–17] Due to the chance of 20% dropouts, a decision was made to enrol 55 patients.

2.8. Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 21.0 (IBM Corp, New York, NY, USA) was used for statistical analysis. Nature of data was determined by applying Kolmogorov-Smirnov (K-S) test. Quantitative values were expressed as mean ± SD /median (IQR) and categorical statistics as frequency (%) based on the nature of data. Percentages of resveratrol and placebo groups were derived by formula: (Mean change at 24 week - Baseline mean)/Baseline mean x 100. Mean changes from baseline ± standard error were calculated for within-group changes and estimated effect size (mean difference = MD) with 95% confidence interval (CI) was reported for between-group differences. Comparison of baseline values between resveratrol and placebo groups for quantitative and categorical variables was done by applying independent t-test/Man-Whitney U test and chi-square tests respectively. Within resveratrol and placebo groups, a comparison of baseline and post-treatment data was done by paired t-test. Analysis of covariance (ANCOVA) was used to compare the post-treatment means of the

two groups. Baseline values were entered as covariate and post-treatment data was taken as an outcome. Statistical significance criteria was a two-tailed p-value ≤ 0.05.

3. Results

3.1. Baseline demographic and biochemical data

Out of the 110 recruited patients, 94 patients completed the study comprising of 45 in resveratrol and 46 in the placebo group (Fig. 1). Final data calculation was performed on a total of 110 patients using the intention to treat (ITT) analysis. The baseline anthropometric and biochemical characteristics of both groups are shown in Table 1. No significant difference was found between the baseline characteristics of both groups.

3.2. Primary and secondary endpoint variables

Within the resveratrol group, a comparison of glycemic control variables at 24 weeks vs baseline revealed a significant reduction in FPG (5.97%), HbA1c (5.60%), fasting insulin (8.59%) and HOMA-IR (13.93%) (Fig. 2). Moreover, the reduction in microalbuminuria (15.65%) was also statistically significant at 24 weeks vs baseline. Regarding lipid profile, 24 weeks intervention in comparison with baseline revealed non-significant modification in total cholesterol (+1.31%), triglycerides (−3.59%), LDL (+2.61%) and HDL (−1.12%) levels. Inflammatory and oxidative stress variables including hs-CRP (11.94%), IL-6 (13.73%), TNF-α (12.70%) and MDA (8.72%) were significantly reduced ($p < 0.05$) after 24 weeks' intervention as compared with baseline (Fig. 3). In the placebo group, all these parameters showed non-significant change.

Between-group comparison revealed that 24 weeks supplementation of resveratrol resulted in significant reduction in FPG (7.56%), HbA1c (6.31%), fasting insulin (9.96%), HOMA-IR (17.96%), hs-CRP (13.12%), TNF-α (13.67%), IL-6 (13.27%) and MDA (8.46%), microalbuminuria (13.48%) ($p < 0.05$ for all) compared to the placebo (Table 2 & 3). However, the comparison of total cholesterol (+1.31%), triglyceride (−3.59%), LDL-C (2.07%) and HDL-C (1.10%) levels between resveratrol and placebo group was non-significant. Their MD±CI have been shown in Table 2.

Results showed 2 fold down regulation of miRNA-34a-5p, miRNA-375–3p, miRNA-21–5p and miRNA-192–5p and 2 fold up regulation of miRNA-126–3p and miRNA-132–3p after 6 months of treatment in resveratrol group ($p \leq 0.05$) as compared to placebo group (Table 4).

Table 1
Baseline demographics and biochemical characteristics levels of the study participants of Resveratrol group (n = 110).

Variables	Resveratrol (n = 55)	Placebo (n = 55)	p-value*
Male n (%)Female n (%)	31(56)24(44)	36(65)19(35)	0.217
Age (years)	49.42 ± 9.04	50.02 ± 12.57	0.774
Weight (kg)	84.38 ± 11.74	83.49 ± 8.02	0.643
Height (m)	1.71 ± 0.08	1.71 ± 0.08	0.922
Body mass index (kg/m^2)	28.89 ± 4.04	28.51 ± 2.65	0.570
Waist Circumference (inches)	37.58 ± 3.06	38.69 ± 4.11	0.112
Systolic BP (mmHg)	139.73 ± 6.34	138.36 ± 8.56	0.344
Diastolic BP (mmHg)	90.27 ± 6.27	88.55 ± 6.71	0.166
Duration of Diabetes (years)	7.33 ± 3.40	8.47 ± 4.61	0.141
Fasting Glucose (mmol/L)	7.37 ± 1.19	7.65 ± 1.68	0.313
Glycosylated hemoglobin (%)	8.64 ± 1.34	8.40 ± 1.15	0.316
Fasting Insulin (mIU/L)	16.14 ± 2.77	16.43 ± 3.88	0.652
HOMA-IR	5.30 ± 1.35	5.65 ± 2.16	0.316
Total Cholesterol (mmol/L)	5.39 ± 0.92	5.58 ± 0.72	0.252
Triglyceride (mmol/L)	2.25 ± 0.72	2.16 ± 0.91	0.570
HDL-Cholesterol (mmol/L)	0.90 ± 0.24	0.90 ± 0.21	1.000
LDL-Cholesterol (mmol/L)	3.47 ± 1.06	3.69 ± 0.79	0.215
hs-C Reactive Protein (mg/L)	3.56 ± 0.76	3.38 ± 0.73	0.212
Interleukin-6 (pg/ml)	15.88 ± 2.51	15.17 ± 3.95	0.263
Tumor necrosis factor-α (pg/ml)	9.98 ± 2.07	9.37 ± 2.99	0.213
Malondialdehyde (μmol/L)	3.67 ± 0.82	3.82 ± 0.68	0.328
Microalbuminuria (mg/mmol)	4.62 ± 0.85	4.62 ± 1.18	0.996
miRNA-34a (ΔCq)	2.71 (1.74–3.57)	2.46 (2.05–3.23)	0.509
miRNA-375 (ΔCq)	2.47 (1.96–2.94)	2.51 (1.96–3.36)	0.593
miRNA-21 (ΔCq)	2.50 (1.94–3.03)	2.24 (1.89–3.00)	0.096
miRNA-192 (ΔCq)	2.22 (1.79–3.41)	2.45 (1.80–4.03)	0.235
miRNA-126 (ΔCq)	4.29 (3.43–5.98)	4.55 (3.77–5.20)	0.793
miRNA-132 (ΔCq)	4.90 (3.68–5.90)	4.43 (3.81–5.58)	0.520
Oral Hypoglycemic Agents; n(%)	10(18)1(2)17	9(16)1(2)14	0.983
Metformin Glimepiride	(31)10(18)14	(25)13(24)14	
Metformin+Vildagliptin	(25)1(2)2(4)	(25)2(4)2(4)	
Metformin+Gliclazide			
Metformin+ Glibenclamide			
Vildagliptin+Gliclazide			
Vildagliptin+Glibenclamide			

Data are expressed as n(%), mean±SD (standard deviation) or median(IQR; interquartile range). HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; hs-C Reactive protein: High sensitive-C Reactive protein; miRNA: microRibonucleic Acid; ΔCq: Delta Quantification cycle. *Statistical analysis was performed by chi-square test/fisher's exact test, independent samples t-test or Mann-Whitney U test

3.3. Safety

There were no significant major and minor adverse effects reported by any patient who participated in the study.

4. Discussion

In this study we have evaluated the effects of 200 mg resveratrol along with recommended

OHGA in patients of T2DM. After 24 weeks of supplementation, resveratrol showed a significant decrease in HOMA-IR and insulin levels. Its effect on the reduction of fasting glucose and HbA1c was significant but less potent. Resveratrol proved its effective anti-inflammatory and anti-oxidative role through a significant decrease in levels of hs-CRP, IL-6, TNF-α and MDA. Resveratrol supplementation also significantly

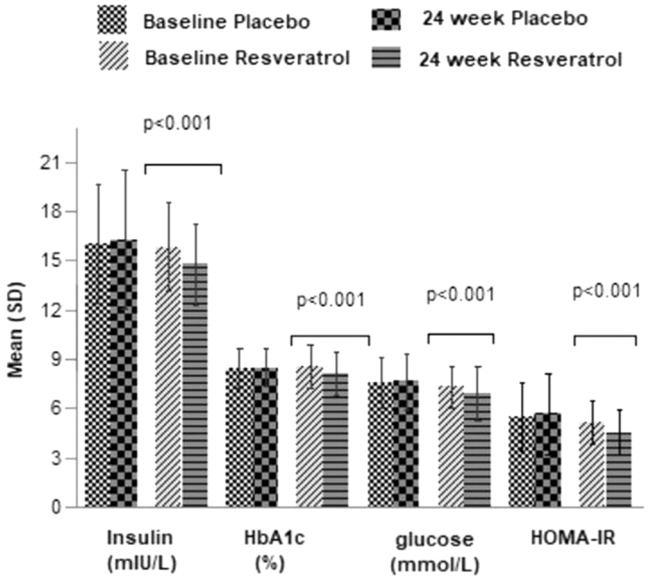

Fig. 2. Without-group changes in glucose parameters in resveratrol and placebo groups from baseline to 24 weeks(n = 110).

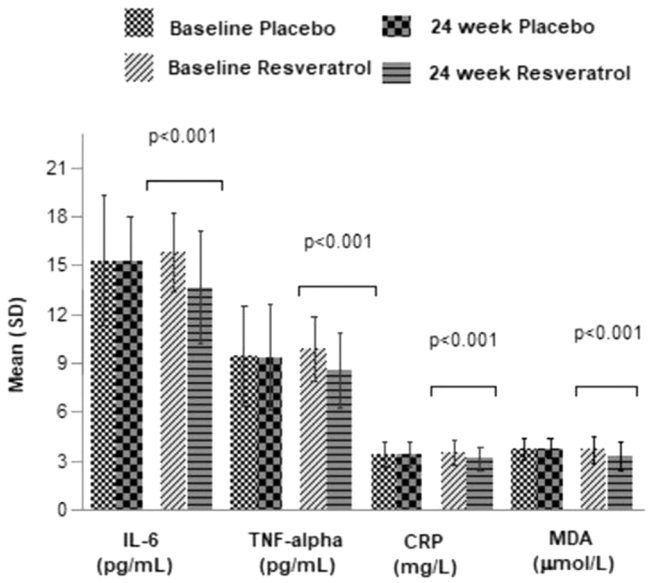

Fig. 3. Without-group changes in inflammatory and oxidative stress markers in resveratrol and placebo groups from baseline to 24 weeks(n = 110).

Table 2
Within-group and between-group changes in glucose parameters, lipid profile and microalbuminuria in resveratrol group from baseline to 24 weeks (n = 110).

Variables	*Change from baseline to 24 weeks		Between groups at 24 week	* ** *p-value
	Resveratrol (n = 55)MC (SE)	Placebo (n = 55)MC (SE)	* **Mean difference (95% Confidence interval)	
FPG (mmol/L)	-0.41(0.14)* *	0.07(0.04)	-0.50(−0.94 to −0.06)	0.016
HbA1c (%)	-0.44(0.13)* *	0.02(0.10)	-0.45(−0.88 to −0.02)	0.033
Insulin (mIU/L)	-1.07(0.26)* *	0.22(0.21)	-1.31(−2.24 to −0.38)	0.001
HOMA-IR	-0.61(0.15)* *	0.18(0.11)	-0.83(−1.37 to −0.29)	0.001
Total-C (mmol/L)	0.06(0.14)	-0.03(0.06)	-0.01(−0.29 to0.32)	1.000
TG (mmol/L)	-0.05(0.07)	-0.02(0.02)	-0.02(−0.27 to 0.22)	1.000
HDL-C (mmol/L)	-0.01(0.01)	-0.01(0.01)	-0.01(−0.06 to 0.06)	1.000
LDL-C (mmol/L)	- 0.10(0.14)	-0.01(0.05)	0.02(−0.29 to 0.34)	1.000
MAU (mg/mmol)	-0.74(0.09)**	-0.07(0.17)	-0.67(−1.11 to0.22)	0.001

Data are expressed as mean change; MC (standard error; SE). FPG: Fasting plasma glucose; HbA1c: Glycosylated haemoglobin; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; Total-C: Total Cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; hs-CRP: High sensitive-C reactive protein; IL-6: Interleukin-6; TNF-α: Tumor necrosis –alpha; MDA: Malondialdehyde; MAU: Microalbuminuria; *change from baseline to 24 weeks within the same group by paired t-test; * * p-value≤ 0.05 (within the same group) by paired t-test, * ** mean difference; MD (95% confidence interval; CI) by analysis of covariance adjusted for baseline, * ** *p-value (between-groups) by analysis of covariance adjusted for baseline.

Table 3
Within-group and between-group changes in inflammatory and oxidative stress markers in the resveratrol group from baseline to 24 weeks (n = 110).

Variables	*Change from baseline to 24 weeks		Between groups at 24 weeks	* ** *p-value
	Resveratrol (n = 55) MC (SE)	Placebo (n = 55) MC (SE)	* **Mean difference (95% Confidence interval)	
hs-CRP (mg/L)	-0.37(0.05)* *	0.01(0.04)	-0.35(−0.70 to −0.01)	0.046
IL-6 (pg/ml)	-2.17(0.45)* *	0.06(0.24)	-1.99(−3.29 to −0.69)	0.001
TNF-α (pg/ml)	-1.33(0.15)* *	0.05(0.12)	-1.25(−1.90 to −0.61)	0.001
MDA (µmol/L)	-0.35(0.07)* *	0.004(0.01)	-0.36(−0.61 to −0.11)	0.001

Data are expressed as mean change; MC (standard error; SE). hs-CRP: High sensitive-C reactive protein; IL-6: Interleukin-6; TNF-α: Tumor necrosis –alpha; MDA: Malondialdehyde; Mau: Microalbuminuria; *change from baseline to 24 weeks within the same group by paired t-test; * * p-value≤ 0.05 (within the same group) by paired t-test, * ** mean difference (95% CI) by analysis of covariance adjusted for baseline, * ** *p-value (between-groups) by analysis of covariance adjusted for baseline.

regulate diabetes-associated miRNA levels.

In our study, resveratrol supplementation for 24 weeks significantly reduces the levels of FPG, fasting insulin, HOMA-IR and HbA1c in the treatment group as compared to placebo. Similarly, a systematic review and meta-analysis conducted in 2017 revealed a significant decline in levels of fasting glucose, HOMA-IR and fasting insulin. Levels of Hba1c were not remarkably changed. As in our study dose of resveratrol was 200 mg/day, this meta-analysis also proposes the cut-off level of more than 100 mg/day of resveratrol for the reduction in fasting blood glucose level.[18] Likewise another placebo-controlled RCT demonstrated the significant decline in fasting glucose, insulin, HbA1c and HOMA-IR levels after intake of 1 g/day of resveratrol for 45 days.[19] A study conducted in Iran revealed that intake of resveratrol significantly decreases the levels of fasting glucose in diabetic patients. Contrary to our study, levels of fasting insulin, HOMA-IR and HbA1c were non-significantly affected after 8 weeks of supplementation in an Iranian study.[20] Similarly another study, conducted for 6 months did not find out any significant change in biochemical markers including fasting glucose, insulin, HOMA-IR and HbA1c levels between resveratrol and placebo groups. [21] In type 2 diabetic women, supplementation of resveratrol along with aerobic exercise significantly reduces the FPG. [22] Another study signifies the beneficial role of resveratrol in diabetic patients by the significant reduction in levels of HbA1c. [23] In a double-blind clinical trial, supplementation of resveratrol in diabetic patients, significantly reduces the serum insulin and HOMA-IR levels. But no significant treatment effect was noticed on fasting blood glucose. [24] In another study, supplementation of resveratrol demonstrated a significant reduction in fasting glucose, serum insulin and HOMA-IR levels. There was no significant effect was noted on HbA1c levels. [25] Our study showed a non-significant change in total cholesterol, triglyceride, LDL-C and HDL-C levels both in the resveratrol and placebo group. These findings are confirmed by meta-analysis and systematic review, results revealed that resveratrol does not have any effect on lipid profile including total cholesterol, LDL-C, HDL-C and triglyceride. [26] In a double-blind clinical trial conducted in diabetic patients, the effect of resveratrol was analyzed on serum triglyceride levels. After 4 weeks of supplementation, no therapeutic effects were observed in experimental and placebo groups. [24] In another randomized trial, patients were either given resveratrol 40 mg or 500 mg/day. No significant beneficial effects were observed in either arm or placebo groups. [21] A recently conducted randomized trial on obese diabetic patients, revealed a significant

Table 4
Comparison of fold changes between resveratrol and placebo group (n = 110).

miRNAs	Resveratrol (n = 55)		Placebo (n = 55)		p-value* *
	Fold change ($2^{-\Delta\Delta Cq}$) Median (IQR)	Fold change due to treatment	Fold change ($2^{-\Delta\Delta Cq}$) Median (IQR)	Fold change due to treatment	
miRNA-34a-5p	0.39(0.37–0.42)	-2.56 *	1.03(0.94–1.12)	1.03	0.001
miRNA-375–3p	0.44(0.40–0.50)	-2.27 *	1.03(1.02–1.06)	1.03	0.001
miRNA-21–5p	0.36(0.35–0.38)	-2.78 *	1.07(1.03–1.13)	1.07	0.001
miRNA-192–5p	0.42(0.38–0.45)	-2.38 *	1.01(0.96–1.04)	1.01	0.001
miRNA-126–3p	2.55(2.51–2.58)	2.55	1.05(0.99–1.09)	1.05	0.001
miRNA-132–3p	2.50(2.39–2.57)	2.50	1.01(0.98–1.04)	1.01	0.001

IQR: Interquartile range; miRNA: micro ribonucleic acid. *Negative fold change due to treatment (fold change reduction) was calculated by formula = −1/fold change ($2^{-\Delta\Delta Cq}$). * *Statistical analysis was performed by Mann-Whitney U test.

increment in HDL-C levels after taking micronized trans-resveratrol. There were no significant effect observed on total cholesterol, LDL-C and triglyceride levels. [25] One possible explanation of improvement in HDL-C levels can be a beneficial effect of resveratrol in obesity. [27] Although in our trial, patients were overweight, the effect of resveratrol on lipid profile was non-significant. A systematic review and meta-analysis conducted in China in 2019 revealed that long term supplementation of resveratrol in obese people significantly decreases triglyceride levels. But at the same time, significant increment in total cholesterol and LDL-C levels were observed in patients besides taking lipid-lowering drugs. [28] These contradictory findings show that resveratrol treatment can be affected by different factors like dose, duration, type of supplement given along disease condition of the study population.

In the present study, levels of microalbuminuria were significantly decreased after intake of resveratrol supplement. By these results, a randomized trial conducted in Iran, revealed a significant reduction in albuminuria in diabetic patients after taking resveratrol.[17] Possible explanation of this reno-protective effect of resveratrol can be its anti-inflammatory action along with improvement in hyperglycemic memory effect. [29]

In our study, resveratrol revealed a significant anti-inflammatory effect by demonstrating a marked reduction in IL-6 and TNF-alpha levels followed by hsCRP. A meta-analysis demonstrated a significant reduction in levels of IL-6 and TNF-α along with a non-significant change in CRP. [30] A [35,36] recent meta-analysis concluded that resveratrol has a significant reduction effect on CRP levels.

According to meta-analysis, its effect on TNF-alpha and IL-6 are non-significant. [15] This meta-analysis recommended further clinical trials to determine the effect of resveratrol on inflammation with different factors like age, obesity and gender. In a randomized trial conducted on diabetic patients, resveratrol supplementation for 4 weeks revealed the beneficial treatment effect on serum IL-6 levels. [31]

In the present study, resveratrol significantly decreases the MDA levels. By our results, another study conducted in Iran revealed a significant treatment effect of resveratrol along with exercise on MDA levels in the female with metabolic syndrome. [32] Inhibition of sirtuin 1 (Sirt-1) is a possible suggestive mechanism responsible for the anti-oxidative effects of resveratrol. [33] In an RCT, administration of resveratrol, although demonstrated the significant anti-oxidative effect by enhancing total anti-oxidant status. But the effect of resveratrol on MDA was non-significant. [34]

Few animal-based studies found the regulatory effect of resveratrol in diabetes through modulation of miRNAs expressions.Regarding RCTs' in diabetic human beings to explore the effectiveness of resveratrol on miRNA expression, data is limited. In 2013 effect of resveratrol on micro RNAs was studied in hypertensive male having T2DM. Supplementation of grape extract containing resveratrol for one year showed the down regulatory expression of pro-inflammatory cytokines along with different micro RNAs (miR-21, miR-181b, miR-663, miR-30c2, miR-155 and miR-34a). [37] Present study confirmed these findings by demonstrating the downward expression of miRNA21 and miRNA 34a after taking 6 months of supplementation of resveratrol. Attenuation of miRNA-21 by resveratrol was found to effective in oxidative stress by decreasing the hydrogen peroxide driven reactive oxygen species production and process of glycolysis in pancreatic stellate cells. [38] Down-regulation of miRNA-34a through resveratrol effectively up-regulate the expression of Sirt-1. [39]

Sirt-1 was studied to have a protective role in reducing oxidative stress and inflammation in T2DM. [40] In the present study, the first time it was proved that resveratrol significantly decreases the expression of miRNA-375. We did not find any study signifying the treatment response of resveratrol on miRNA-375 in T2DM. This necessitates more clinical trials to further explore this beneficial aspect of resveratrol. Down regulation of miRNA-375 may be helpful in diabetes mellitus by maintaining sufficient insulin secretion in the body through a reduction in apoptotic loss of beta cells of the pancreas. Activation of the cAMP-PKA signalling pathway was reviewed to be responsible to decrease the beta cells apoptosis through decreased expression of miRNA-375. [41] In our study, miRNA-192 levels were also significantly down-regulated by supplementation of resveratrol. Data indicating the direct effect of resveratrol on this miRNA in human diabetic patients is still lacking. The literature reviewed the importance of miRNA-192 in diabetic kidney disease by discussing the possible role of Smad, p53, Ets-1 and β-catenin-interacting protein expression. [42] Resveratrol was found to be effective in various chronic diseases by modulating the expression of these proteins. [43]

Thus future studies to explore the treatment effect of resveratrol on miRNA-192 may be helpful to control the T2DM associated kidney disease development and progression.

To date, no other research is available to indicate the therapeutic potential of resveratrol on miRNA-126 and miRNA-132 in human patients of T2DM. In the present study, resveratrol supplementation significantly up-regulated the expression of miRNA-126 and miRNA-132. The findings of our trial confirm the results of the study in which resveratrol was found to enhance the expression of miRNA-126 in mouse insulinoma cell line Min6. Here resveratrol amends the uric acid induced pancreatic beta-cell damage and elevates the insulin secretion through PI3K/AKT signal pathways.[44] Effectiveness of resveratrol in reducing oxidative stress and inflammation in the body through up-regulation of miRNA-126 levels may also be associated with attenuated expression of an [45,46]inflammation-associated protein; HMGB1.

Impaired angiogenic effects of miRNA-126 and miRNA-132 in T2DM were revealed through the augmented expression of antiangiogenic EVH1 domain-containing protein 1 (Spred1) & RASA1 or p120RasGAP and reduction in the expression of proangiogenic vascular endothelial growth factor. [47] Up-regulated expression of miRNA-132 also attenuates inflammation associated NF-kB, TNF-alpha, NOD-like receptor and toll-like receptor signalling pathways. [48]

4.1. Study limitations

Firstly, the exact assessment of compliance and level of absorption depending upon the analysis of plasma concentration of resveratrol. It

was not carried out in the present study due to limited resources. Secondly, it was a single-centre study. That's why the generalizability of findings may be limited.

5. Conclusion

Resveratrol supplementation improves glycemic control by showing a maximum decrease in HOMA-IR and insulin levels. Change in FPG and HbA1c was although significant but percentage reduction was very less. Resveratrol also shows a significant decline in inflammation and oxidative stress. microRNA expression was also significantly improved. There was no major or minor side effect observed during the study. Thus, supplementation of resveratrol may be useful in diabetic patients on oral hypoglycemic agents for the prevention of T2DM progression. Further multicenter clinical studies are required to validate the findings of this research and to explore the molecular mechanisms of resveratrol through next-generation sequencing on messenger RNAs and miRNAs pathway analysis.

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Conflict of interest

The authors here certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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