Life Sciences 258 (2020) 118155

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Novel therapeutic intervention of coenzyme Q10 and its combination with pioglitazone on the mRNA expression level of adipocytokines in diabetic rats

Rajesh A. Maheshwari, Ghanshyam R. Parmar*, Denish Hinsu, Avinash K. Seth, Ramachandran Balaraman

Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, At Post Piparia, Taluka: Waghodia, Dist: Vadodara, Vadodara-391760, Gujarat, India

ARTICLE INFO

Keywords:
Adipocytokines
Antioxidant enzymes
Coenzyme Q10
Pioglitazone and mRNA expression
White adipose tissue

ABSTRACT

Aims: Aim of the present study was to investigate the effect of co-administration coenzyme Q10 and pioglitazone on the mRNA expression of adipocytokines in white adipose tissues of chemically induced type 2 diabetes mellitus in rats.

Main methods: Diabetes was induced by administration of streptozotocin (65 mg/kg, i.p.), followed by nicotinamide (110 mg/kg, i.p.) 15 min later. The diabetic rats were treated coenzyme Q10 (Q10, 10 mg/kg, p.o.) or pioglitazone (PIO, 20 mg/kg, p.o.) alone and their combination for four weeks. Biochemical parameters like FBS level, insulin and HbA1c along with tissue levels of MDA, SOD, CAT and GSH were estimated. The mRNA levels of ADIPOQ, RBP4, RETN, IL-6 and TNF-α in White Adipose Tissue (WAT) were measured.

Key findings: Treatment with Q10 + PIO showed a significant reduction in the levels of FBS, HbA1c and a significant increase in insulin levels as compared to normal control group. Additionally, there was a significant change in the levels of biomarkers of oxidative stress after treatment with Q10 + PIO as compared to streptozotocin-nicotinamide group. Treatment with Q10 + PIO also significantly altered the mRNA expression of ADIPOQ, RETN, IL-6 and TNF-α when compared to monotherapy. However, mRNA expression of RBP4 did not alter in Q10 + PIO treated animal as compared to Q10 or PIO alone.

Significance: It is concluded that co-administration of Q10 and PIO has been shown the better therapeutic effect on the mRNA expression of adipocytokines and oxidative stress parameters as compared to either Q10 or PIO.

1. Introduction

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia due to insufficient insulin secretion and/or action. The primary component incites insulin resistance in type 2 diabetes mellitus (T2DM) are glucotoxicity, lipotoxicity, oxidative stress, inflammation, endoplasmic reticulum stress and amyloid deposition in the pancreas [1–3].

Hyperglycemia-induced oxidative stress in terms of an increased generation of reactive oxygen/nitrogen species (ROS/RNS) and concealment of antioxidant defenses, such as SOD, CAT, GPx, play vital roles in the pathogenesis of DM and its complications [4–6].

Adipose tissue plays a vital role in insulin sensitivity and energy expenditure [7] and dysfunction in adipocytes is related with insulin resistance and T2DM [8]. Adipocytes secrete various adipocytokines such as adiponectin, resistin and inflammatory cytokines. Increased levels of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β and resistin as well as reduced leptin, visfatin and adiponectin can worsen insulin resistance [8–10]. Chronic inflammation is one of the other condition that seem to take part in the pathogenesis of T2DM [11]. Additionally, it is accounted for those systemic etiological components, such as an abdominal obesity and insulin resistance, lead to incitement of inflammation in T2DM [12].

It is well known that the incidence of diabetes mellitus is very high throughout the world. It is a well-known fact that chronic diabetes is capable of inducing complications like kidney, retinal and nerve damage which is collectively called nephropathy, retinopathy and neuropathy, respectively [13–15]. It was previously reported that free radical generations (oxidative stress) are the main cause of several disorders including the complications of diabetes. This oxidative stress is ultimately responsible for nephropathy, retinopathy, neuropathy and other cardiovascular diseases [16,17].

It was stated that there is increasing evidence in the defensive impact of dietary antioxidants as potential adjuvant treatment to prevent

* Corresponding author at: Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, At Post Piparia, Taluka: Waghodia, Dist: Vadodara, Vadodara-391760, Gujarat, India.
E-mail address: ghanstaurus22@gmail.com (G.R. Parmar).

https://doi.org/10.1016/j.lfs.2020.118155
Received 18 May 2020; Received in revised form 15 July 2020; Accepted 23 July 2020
Available online 28 July 2020
0024-3205/

or postpone diabetic complications [18,19]. The 2,3 dimethoxy-5 methyl-6-decaprenyl benzoquinone, i.e. coenzyme Q10 (Q10) or ubiquinone is a nutrient -like substance which is lipid soluble in nature and hydrophobic inside of the phospholipid bilayer of the cell membrane. It exists in a wide range of dietary food including meat, fish, vegetable oils, and nuts [20]. It has been recently detailed that Q10 is an effective anti-inflammatory activity [21], antiulcer [22] and antioxidant [23]. The mechanism of action of Q10 to improve insulin sensitivity was comprised of modulation in insulin and adiponectin receptor as well as tyrosine kinase (TK), phosphoinositide 3-kinase (PI3K), glucose transporters, along with improving lipid profile, redox system, receptor for advanced glycation end products (sRAGE), and adipocytokines [22]. The investigation likewise focuses to the potential positive outcome of Q10 as an add-on to conventional antidiabetic therapies.

Thiazolidinediones (TZD), including pioglitazone (PIO), ameliorates insulin resistance by binding to and activating PPARγ (peroxisome proliferator-activated receptor-γ) in adipose tissues [24]. PPARγ is exceptionally expressed in white adipose tissue (WAT) with lower levels in skeletal muscle and liver [25]. PPARγ activators are important agents of adipogenesis, improve insulin sensitivity and dyslipidemia, and rise body weight [26]. The TZDs manage different genes involved in adipocyte differentiation or lipid and glucose homeostasis. TZDs are likewise known to adjust different adipokines [27–29].

Since Q10 was shown to produce some positive effect on insulin sensitivity, we were interested to see whether combined treatment with Q10 and PIO has outcome by virtue of antioxidant effect. Therefore the present work was planned to investigate the co-administration of PPRγ agonist and Q10 to show whether such combination have any synergistic effect on the fasting blood glucose (FBG) level, antioxidant enzyme as well as mRNA expression of adipocytokines levels in T2DM.

2. Material and method

2.1. Chemicals

Streptozotocin (STZ)-Nicotinamide (NA) were obtained from Himedia, Mumbai, India. Pioglitazone was obtained as a gift sample from Cadila Pharmaceutical Pvt. Ltd., Ahmedabad, India. Coenzyme Q10, 3-(N-morpholino) propane sulfonic acid (MOPS), ethidium bromide (EtBr), PBS buffer and chloroform were purchased from Sigma-Aldrich, USA. RNaseZap was purchased from Invitrogen, USA. RNA later solution, Qiazol reagent, lithium chloride, and RNase free water were purchased from Qiagen, Germany. Formaldehyde, formalin, and 2-mercaptoethanol were procured from LobaChem Pvt. Ltd., Mumbai, India. Agarose was obtained from Lonza, USA. Heparin, xylazine, ketamine, and normal saline were purchased from a local vendor of Vadodara, Gujarat, India.

2.2. Animals

Healthy male Wistar albino Rats (180–200 g, 12–13-week-old) obtained from Zydus Research Center, Ahmedabad, Gujarat, India. They were allowed have free access food and drinking water. They were maintained under constant temperature (25 ± 1 °C), 35–60% humidity and 12-hour light and dark cycle. The study was approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethical Committee (IAEC) of Sumandeep Vidyapeeth, Vadodara, Gujarat, India (Approval no.: SVU/DP/IAEC/2016/05/26).

2.3. Induction of T2DM

The T2DM was induced in an overnight fasted animal by single intraperitoneal (i.p.) injection of STZ (65 mg/kg, dissolved in citric buffer pH 4.5), followed by NA (110 mg/kg, i.p., dissolved in normal saline) [30]. Hyperglycemia was confirmed by FBG level, estimated at 72 h and then on 7 days after injection of STZ-NA using Accucheck glucometer (Roche, USA). An animal showing the FBG level > 200 mg/dL were considered as diabetic animals in the experiment (A week after STZ-NA administration was considered as 0 week or initial stage in this study).

2.4. Experimental design

The diabetic rats were randomized into different groups ($n = 6$) as follow:

Group 1:	Normal control (NC)
Group 2:	STZ-NA group
Group 3:	Q10 (10 mg/kg, p.o.)
Group 4:	PIO (20 mg/kg, p.o.)
Group 5:	Q10 + PIO

T2DM was initiated in all groups except group 1. All aforementioned treatments were started one week after injection of STZ-NA and continued for 28 days. The NC and STZ-NA rats received 1% w/v gum acacia (1% w/v) solution orally, while Q10 and PIO suspended in 1% (w/v) gum acacia. On the 28th day, blood samples were withdrawn by puncturing the retro-orbital under light anesthesia and stored with or without EDTA tubes. For separation of serum, blood was allowed to clot for 15 min, and it was then centrifuged at 5000 rpm for 20 min. The serum was stored at −20 °C until further biochemical analysis [31].

2.5. Body weight

Body weight was measured on 7th day of the induction of diabetes by STZ and nicotinamide, all the treatments will be given orally as mentioned in the respective groups for 4 weeks and this day will be considered as day 1. The body weight on 1st and 28th day of will be measured.

2.6. Estimation of serum insulin and HbA1c

Serum insulin (ERINS, Invitrogen, USA) was measured by ELISA microplate reader (iMax, Biorad, USA) according to the manufacturer's protocol. HbA1c was measured from whole blood using D10 (Biorad, USA).

2.7. Biomarker of oxidative stress in the tissue

The oxidative stress parameters were estimated by in both liver and kidney tissues. The tissue levels of malondialdehyde (MDA content), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were estimated as biomarkers of oxidative stress. Briefly, after sacrificing the animal, liver and kidney were transferred to ice-cold phosphate buffered saline pH 7.4. The liver and kidney were cross-chopped with a surgical scalpel into fine slices, suspended in cold 0.25 M sucrose solution. The tissues were then homogenized in cold Tris-HCL buffer (10 mM, pH 7.4) and centrifuged at 6000 rpm at 4 °C for 20 min using Thermo Scientific ST8R (USA). The clear solution was utilized for the quantification of MDA [32], SOD [33], CAT [34] and GSH [35].

2.8. Gene expression analysis of adipocytokines

2.8.1. Collection of white adipose tissue

At the end of the study, the animals were anesthetized by intraperitoneal injection of xylazine 87 mg/kg and ketamine 13 mg/kg. White adipose tissue (WAT) was collected in RNA later (QiagenGmBH, Germany) solution from rat abdominal cavity and stored at −20 °C

until total RNA was isolated [36].

2.8.2. Isolation of mRNA

The total RNA was isolated using a phenol-chloroform method using RNeasy kit (Qiagen, San Jose, California) with slight modification [37]. Briefly, the frozen tissue sample was washed with PBS to remove RNA later solution. The tissue was homogenized using QiazolLysis reagent (Qiagen, San Jose, California) with D160 homogenizer (Scilogex, Connecticut, United States). The contents were transferred into 1.5 mL tube. Then 20 μL of β-mercaptoethanol (Sigma Aldrich, USA) was added to this tube and vortex for 15 s and incubated for 5 min at room temp. Later, 400 μL of chloroform-isoamyl alcohol mixture (24:1, Sigma Aldrich, USA) per mL of QiazolLysis reagent was mixed in above mixture and vortex for 15 s. The tube was allowed to stand for 3 min at room temp and then centrifuged (Sorvell SR8, Thermo scientific, USA) at 12000 g for 20 min at 4 °C. The upper aqueous phase was separated into a new tube. Further, 200 μL of 10 M Lithium chloride (LobaChem, Mumbai,) was added and mix well. Then 0.5-time cold isopropanol (LobaChem, Mumbai) was added to the above mixture and vortex for 15 s. The mixture was kept at −20 °C for 1 h. Later, the tube was removed from the refrigerator and centrifuged (Sorvell ST8R, Thermo scientific, USA) at 12000*g* for 20 min at 4 °C. The supernatant was carefully aspirated without disturbing the RNA pellet. The pellet was washed two times with 70% ethanol and centrifuged at 12000*g* for 10 min at 4 °C. Traces of ethanol were removed with a pipette and allowed to dry. Further, 70 μL of RNase free water (Qiagen, GmBH Germany) was added to dissolve the RNA pellet and stored in −20 °C.

The quality and quantity of RNA were measured using QIAxpert System (Qiagen, GmBH Germany) by taking the ratio of absorbance at 260 nm and 280 nm. The integrity of total RNA was also confirmed on 1% agarose-formaldehyde gel using MOPS-Formaldehyde (Sigma Aldrich, USA)as an electrophoretic buffer [38] in Mini Submerge gel electrophoresis system (Biorad, USA) and images were documented by Gel Doc™ EZ Gel (Biorad, USA).

2.8.3. Reverse transcription and quantitative real-time PCR

Total RNA (1 μg) was reverse transcribed for synthesis of first strand cDNA using QuantiTect® Reverse Transcription kit (Qiagen, Germany) according to the manufacture's protocol in Veriti® 96-Well Thermal Cycler (Applied Biosystems, USA). Quantitative Real-Time PCR (qPCR) was performed in 7500 Fast qRT-PCR (Applied Biosystems, Foster City, CA, USA) with an equal amount of cDNA from each sample using Quantifast Probe PCR kit (Qiagen, GmBH Germany) according to the manufacturer's protocol. FAM and TAMRA labeled TaqMan probes were utilized to quantify the mRNA expression levels of following adipocytokines: (i) *ADIPOQ*, (ii) *RBP4*, (iii) *RETN* (iv) *IL-6* (v) *TNF-α*. Primers and probes of interested adipocytokines and an endogenous control gene (*ACTB*: β-actin) were designed at exon-exon spanning junction using the NCBI primer blast tool to eliminate gDNA contamination and synthesized from Eurofins (Germany) listed in Table 1. The 7500 Fast RTPCR (Applied Biosystems, USA) was programmed using the 7500 software v2.3 (Applied Biosystems, USA) with PCR reaction condition for denaturation at 95 °C for 3 s, combined annealing and extension at 60 °C for 30 s up to 40 cycles. Gene expression assay of *TNF-α* and *IL-6* was measured using QuantiFast SYBR Green PCR Kit (Qiagen, GmBH Germany) according to manufacturer's protocol. All PCR reaction samples were analyzed in duplicate and the average C_t value were used to calculate the relative quantification using the $^{\Delta\Delta}$Ct method.

3. Statistical analysis

All values are expressed as means ± SEM. Statistical analysis of data was done using one-way ANOVA followed by post hoc Tukey's multiple comparison tests to state the difference within groups in Prism 7, GraphPad Software. The significance level was set at $P < 0.05$ for all tests.

4. Results

4.1. Effect of Q10 and PIO combination on FBG level and body weight in diabetic rats

In STZ-NA induced diabetic group, there was a significant ($P < 0.001$) elevation in FBG level and reduction in body weight on 28th days as compared to the NC animals. Treatment with Q10 or PIO (20 mg/kg) or Q10 + PIO showed a significant ($P < 0.05$, $P < 0.01$, $P < 0.01$) decrease in FBG level as compared to STZ-NA induced diabetic group, respectively. In addition, Q10 or PIO or Q10 + PIO treated rats showed a significant increase in body weight as compared to STZ-NA group. Moreover, treatment with Q10 and PIO did not show any significant changes in FBG level and body weight when compared with monotherapy (Fig. 1A–B).

4.2. Effect of Q10 and PIO combination on serum insulin and HbA1c in diabetic rats

After 4 weeks, STZ-NA induced diabetic rats showed a significant decrease in the level of serum insulin ($P < 0.001$) and increase in HbA1c ($P < 0.001$) level as compared with NC group. However, treatment with PIO or Q10 + PIO showed a significant ($P < 0.001$) increase in serum insulin level as compared to STZ-NA group, while treatment with Q10 or PIO or Q10 + PIO showed a significant ($P < 0.05$, $P < 0.01$, $P < 0.01$) decrease in HbA1c as compared to STZ-NA control. However, there was no significant difference in insulin and HbA1c level in Q10 + PIO treated rats when compared with monotherapy (Fig. 2 A-B).

4.3. Effect of Q10 and PIO combination on MDA, GSH, CAT and SOD in diabetic rats

In the present study, it was shown that a significant ($P < 0.001$) increase in activity of lipid peroxidation resulting in an elevation of the content of MDA, while there was a significant ($P < 0.001$) decrease in the levels of GSH, SOD and CAT in liver and kidney tissues of STZ-NA induced diabetic group as compared to NC group. However, treatment with Q10 or PIO or Q10 + PIO showed a significant decrease in MDA content ($P < 0.001$, $P < 0.01$; $P < 0.05$, $P < 0.05$; $P < 0.001$, $P < 0.001$) in liver and kidney tissues as compared to STZ-NA group, respectively. In addition, GSH ($P < 0.001$, $P < 0.001$; $P < 0.05$, $P < 0.05$; $P < 0.001$, $P < 0.001$), CAT ($P < 0.01$, $P < 0.001$;

Table 1
Primer and labeled probe sequence of adipocytokines analyzed by qPCR.

Gene	Forward sequence (5′ > 3′)	Reverse sequence (5′ > 3′)	Probe	Fragment size (bp)
ADIPOQ	CCACCCAAGGAAACTTGTGC	GACCAAGAACACCTGCGTCT	**6-FAM**-ACAATGGGATACCGGGCCGTGA-**TAMRA**	136
RBP4	ACAAGGCTCGTTTCTCTGGG	TGTGAAAGTGCCCACCATGT	**6-FAM**-TGAGCGCTACAGCCAAGGGA-**TAMRA**	188
RETN	GAGCTCTCTGCCACGTACTTA	TGGCTTCATCCATGGGACAC	**6-FAM**-TTCCTTGTCCTGGGGCTGCT-**TAMRA**	120
IL-6	GCAAGAGACTTCCAGCCAGT	CCATTGCACAACTCTTTTCTCA	–	200
TNF-α	ATGGGCTCCCTCTCATCAGT	GCTTGGTGGTTTGCTACGAC	–	106
ACTB	CTCTGTGTGGATTGGTGGCT	CGCAGCTCAGTAACAGTCCG	**6-FAM**-TCCATCGTGCACCGCAAATGCT-**TAMRA**	139

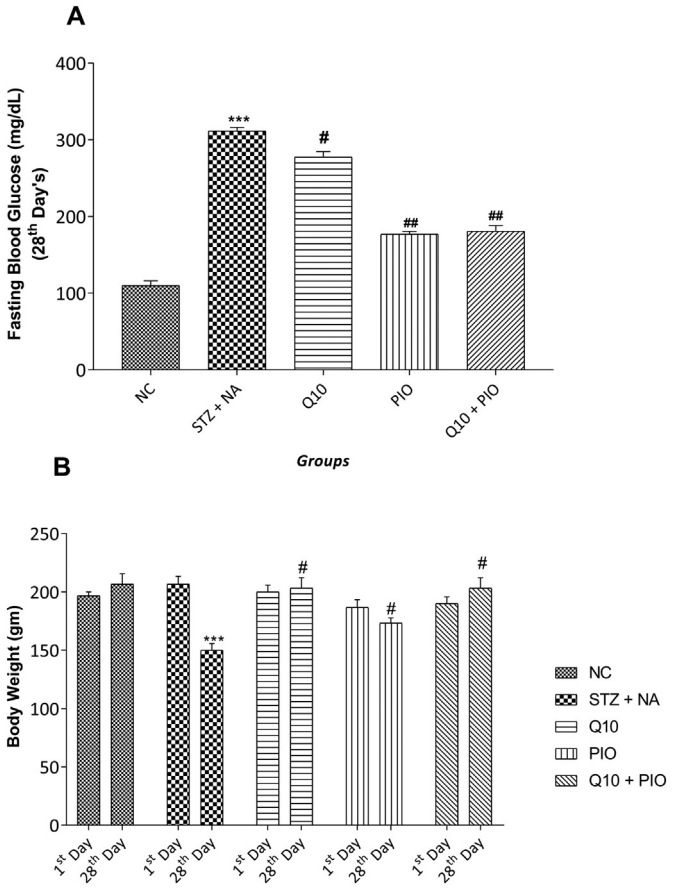

Fig. 1. Effect of Q10 and PIO combination on FBG level and body weight in STZ-NA induced diabetic rats. Values are expressed as Mean ± S.E.M. ($n = 6$). ***$P < 0.001$ compared with NC; #$P < 0.05$, ##$P < 0.01$ compared with STZ-NA.

$P < 0.05$, $P < 0.05$; $P < 0.001$, $P < 0.001$) and SOD ($P < 0.01$, $P < 0.01$; $P < 0.05$; $P < 0.001$, $P < 0.001$) levels were significantly increased in Q10 or PIO or Q10 + PIO treated rats when compared to STZ-NA group, respectively. Moreover, concomitant administration of Q10 with PIO showed a more significant alteration in biomarkers of oxidative stress than monotherapy of Q10 or PIO (Table 2).

4.4. Effect of Q10 and PIO combination on mRNA expression levels of ADIPOQ, RETN, RBP4, TNF-α and IL-6 in WAT of diabetic rats

Relative gene expression profiling of adipocytokines i.e. *ADIPOQ* and *RBP4* along with the pro-inflammatory cytokines like *RETN*, *TNF-α* and *IL-6* was measured as fold change based on the C_t value obtained using qPCR in WAT.

STZ-NA treated rats showed a significant ($P < 0.001$) down regulation in the mRNA expression levels of *ADIPOQ* and up regulation of RETN, RBP4, NF-α and IL-6 as compared to NC group. Treatment with Q10 or PIO or Q10 + PIO showed a significant ($P < 0.05$; $P < 0.01$; $P < 0.01$) up regulation in the mRNA expression levels of *ADIPOQ* in WAT as compared to STZ-NA induced diabetic rats. However, concomitant administration of Q10 with PIO showed a more significant up regulation in the mRNA expression levels of *ADIPOQ* ($P < 0.5$) than Q10 or PIO administered alone.

In addition, administration of Q10 or PIO or Q10 + PIO showed a significant down regulation in the mRNA expression levels of *RETN* ($P < 0.05$; $P < 0.01$; $P < 0.01$), RBP4 ($P < 0.05$; $P < 0.05$; $P < 0.05$), *TNF-α* ($P < 0.05$; $P < 0.01$; $P < 0.01$) and IL-6 ($P < 0.05$; $P < 0.01$; $P < 0.01$) in WAT as compared to STZ-NA

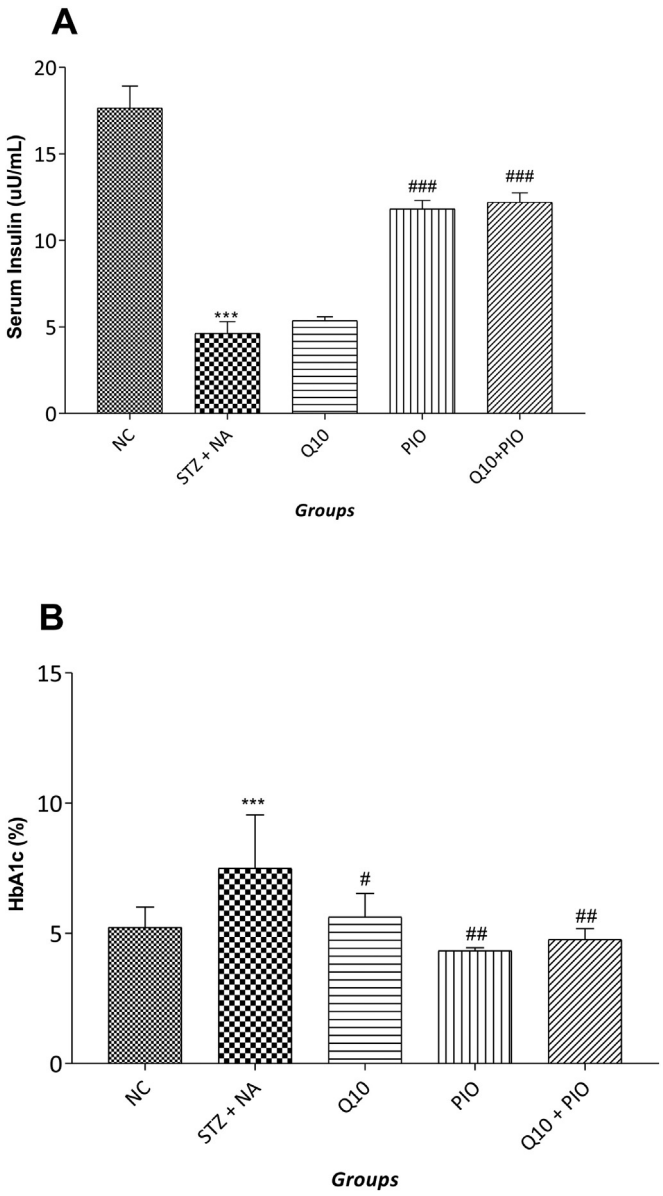

Fig. 2. Effect of Q10 and PIO combination on serum insulin and HbA1c in diabetic rat. Values are expressed as Mean ± S.E.M. ($n = 6$). ***$P < 0.001$ compared with NC; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with STZ-NA.

treated rats. In contrast, co-administration of Q10 with PIO showed a more significant ($P < 0.05$) down regulation in the mRNA expression of RETN ($P < 0.05$), TNF-α ($P < 0.05$) and IL-6 ($P < 0.05$) in WAT when compared to Q10 or PIO alone. In Q10 + PIO treated rats, mRNA expression of RBP4 in WAT was not significantly altered than monotherapy (Fig. 3A–E).

5. Discussion

In the present investigation, an attempt was made to check the effect of PIO alone and its combination with Q10 on FBS, serum insulin, HbA1c and the levels of MDA, SOD, CAT and GSH along with gene expression levels of various adipocytokines like *ADIPOQ*, *RETN*, *RBP4*, *TNF-α* and *IL-6* in WAT in STZ-NA induced diabetic rats.

Induction of diabetes was confirmed with the significant elevation of FBG and percentage of HbA1c in STZ-NA induced diabetic rats this might be due to lack of release of insulin from β-cells or insulin

Table 2
Effect of Q10 alone and its combination with PIO on MDA, GSH, CAT and SOD in diabetic rat.

Groups	MDA (nM/mg)		GSH (pg/mg)		CAT (mM/mg)		SOD (Units/mg)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group 1: NC	0.76 ± 0.05	0.95 ± 0.04	9.86 ± 0.65	10.69 ± 1.21	11.39 ± 0.89	10.36 ± 0.66	7.65 ± 0.68	9.50 ± 0.63
Group 2: STZ + NA	1.51 ± 0.18***	1.71 ± 0.15***	3.97 ± 0.27***	3.96 ± 0.22***	5.56 ± 0.30***	4.467 ± 0.81***	4.79 ± 0.31***	5.26 ± 0.60***
Group 3: Q10 10 mg/kg, p.o.	0.80 ± 0.06$^{\#\#\#}$	1.05 ± 0.06$^{\#\#}$	8.13 ± 0.29$^{\#\#\#}$	7.44 ± 0.40$^{\#\#\#}$	8.88 ± 0.32$^{\#\#}$	8.16 ± 0.61$^{\#\#\#}$	6.63 ± 0.40$^{\#\#}$	7.99 ± 0.10$^{\#\#}$
Group 4: PIO 20 mg/kg, p.o.	0.93 ± 0.04$^{\#}$	1.14 ± 0.08$^{\#}$	6.88 ± 0.19$^{\#}$	6.84 ± 0.32$^{\#}$	7.43 ± 0.66$^{\#}$	6.933 ± 0.32$^{\#}$	5.71 ± 0.46	6.98 ± 0.45$^{\#}$
Group 5: Q10 + PIO	0.81 ± 0.02$^{\#\#\#, \dagger}$	1.02 ± 0.05$^{\#\#, \dagger}$	9.13 ± 0.47$^{\#\#\#, \dagger\dagger, \$}$	8.45 ± 0.51$^{\#\#\#, \dagger, \$}$	9.85 ± 0.44$^{\#\#\#, \dagger, \$}$	8.43 ± 0.31$^{\#\#\#, \dagger}$	7.52 ± 0.22$^{\#\#\#, \dagger, \$}$	8.54 ± 0.30$^{\#\#\#, \dagger, \$}$

Values are expressed as Mean ± S.E.M. ($n = 6$).
*** $P < 0.001$ compared with NC.
$^{\#}$ $P < 0.05$,
$^{\#\#}$ $P < 0.01$.
$^{\#\#\#}$ $P < 0.001$ compared with STZ-NA.
$^{\$}$ $P < 0.05$ compared with Q10.
† $P < 0.05$.
†† $P < 0.01$ compared with PIO.

resistance and glycation of hemoglobin. As per the previous reports it was found that Q10 having antidiabetic effect [39], while in contradictory some the literatures also revealed that Q10 did not have any effect on blood glucose level [40]. In present set up, administration of Q10 or PIO alone and their combination showed a significant decrease in the elevated FBG levels and a significant reduction in percentage of HbA1c. This reduction in the levels of FBG might be due to release of insulin from β-cells or from its bound forms [41]. Generally, in diabetic rats, the elevated percentage of HbA1c might be due to binding of excessive glucose to the hemoglobin [42]. The oral treatment with of Q10 or PIO alone and their combination showed a significant reduction in levels of HbA1c suggesting the positive role in the reduction of complications in diabetes.

Oxidative stress is the manifestation of various cardio vascular complications in diabetes. This might be due to linkage of elevated FBG and auto-oxidation of glucose and shift in the redox balances. The underlying mechanism of oxidative stress is due to the increased ROS which subsequently leads to decrease in tissue concentration of antioxidant enzymes such as GSH, SOD and CAT. However, in the present investigation, STZ-NA administration also produced similar type of effect as shown in the earlier report [43,44]. Treatment with Q10 or PIO or Q10 + PIO showed a significant elevation in the levels of various antioxidant enzymes like SOD, catalase and GSH as compared to STZ-NA diabetic rats. However, the lipid peroxidation (MDA) level was significantly reduced in Q10 or PIO or Q10 + PIO as compared to STZ-NA group. However, the combination therapy of Q10 and PIO showed a more significant alteration in biomarkers of oxidative stress than monotherapy. This alteration in the levels of antioxidant enzymes levels corroborates the antioxidant effect of Q10 along with PIO. This co-administration of Q10 along with PIO might be beneficial in inhibition of oxidative stress and associated complications of diabetes [45].

Abdominal fat is directly associated with obesity, diabetes, CVDs and other metabolic syndromes. Thus, the WAT was chosen to be studied in rats which resembles the visceral fat tissues in human [46]. Adipocytokines secretions from this tissue are transported in portal vein, liver and resulting to the chronic inflammation, IR, and obesity. Thus, the gene expression analysis of ADIPOQ, RETN, RBP4, TNF-α, and IL-6 would give an underlying relationship between T2DM and symptoms of metabolic syndromes [47]. Hence, the present investigation is showing that there is a molecular relationship between antioxidant like Q10 and its combination with antidiabetic drug like PIO on the mRNA expression of various adipocytokines.

Generally, it has been previously established that the mRNA expression of *ADIPOQ* are down regulated in WAT of diabetic rats along with a higher expression of inflammatory cytokines such as RETN, TNF-α and IL-6 [48,49].

The high molecular weight oligomer of adiponectin is the chief form responsible behind the insulin sensitizing action of adiponectin [50]. Thiazolidinedione has been reported to up regulate the expression of adiponectin [49,51]. Statnick et al., have shown that serum adiponectin levels are decreased in patients with type 2 diabetes [52]. The T2DM is associated with insulin resistance, which is ameliorated, in part, by the high circulating levels of endogenous adiponectin, especially the high molecular weight counterpart. Also, adiponectin has been reported to sensitize the body tissues toward actions of insulin [53]. In current examination, STZ-NA treated rats showed similar types of down regulation in mRNA expression of ADIPOQ. Hence, we investigated the effect of Q10 or PIO or Q10 + PIO on the relative gene expression of mRNA in WAT against STZ-NA induced diabetic rats. Our finding showed that the mRNA expression of *ADIPOQ* was up regulated in rats treated with Q10 or PIO Q10 + PIO as compared to STZ-NA group. However, the combination therapy of Q10 and PIO showed a more significant up regulation than Q10 or PIO administered singly.

Mechanistic studies have shown that RBP4 may be involved in several etiologic pathways leading to type 2 diabetes development, such as dysregulation of insulin resistance and insulin secretion,

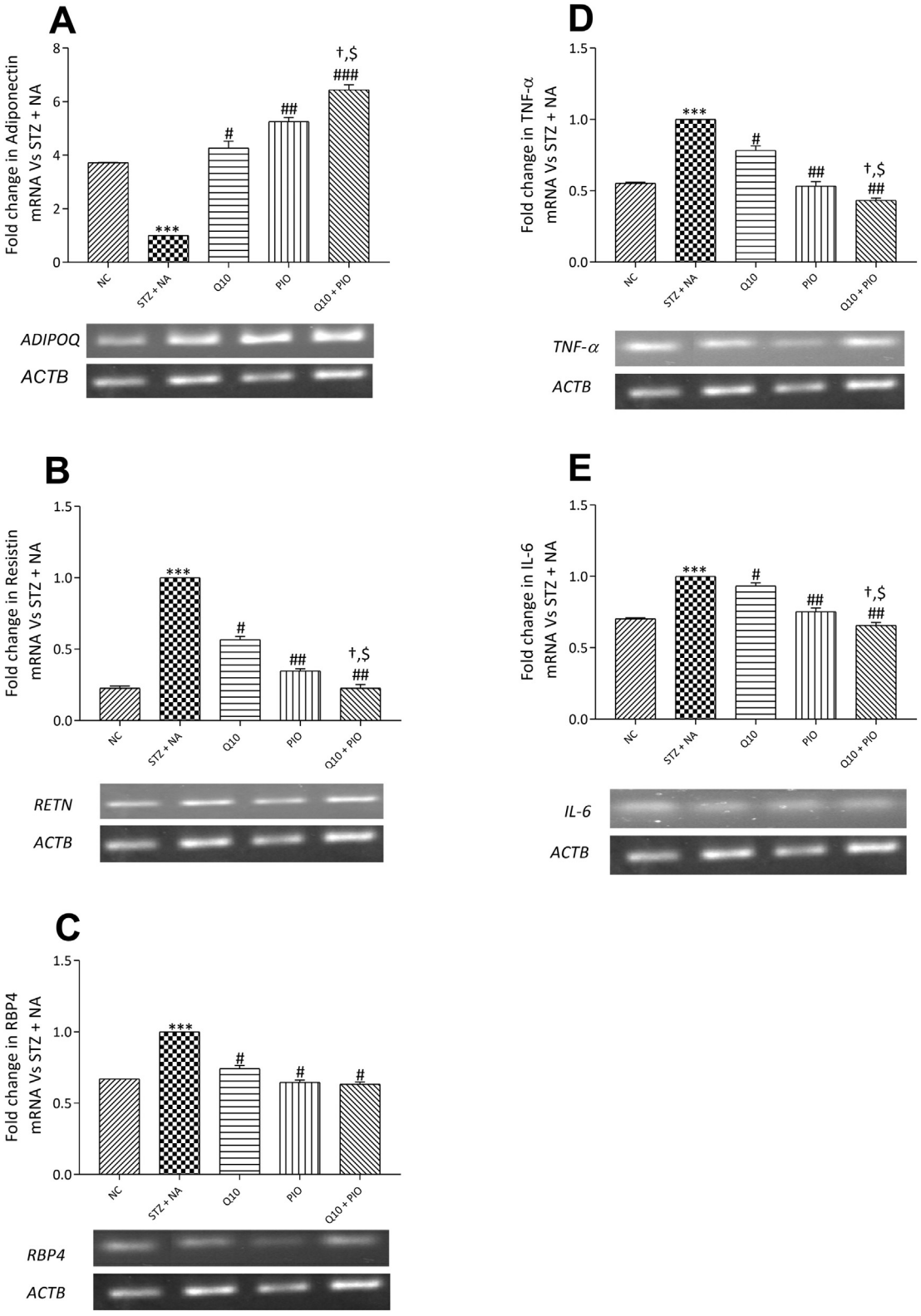

Fig. 3. Effect of Q10 and PIO combination on mRNA expression of (A) *ADIPOQ* (B) *RETN* (C) *RBP4* (D) *TNF-α* and (E) *IL-6* in WAT of STZ-NA induced diabetic rats analyzed by using qPCR. The bars represent fold change in treated group vs. STZ-NA. Values are expressed as Mean ± S.E.M. ($n = 6$). ***$P < 0.001$ compared with NC; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared with STZ-NA; \$$P < 0.05$ compared with Q10; †$P < 0.05$ compared with PIO.

inflammation, and failure of intracellular lipid homeostasis. RBP4 has shown to decrease insulin sensitivity by inducing liver expression of phosphoenolpyruvate kinase [54] or stimulating inflammation in adipocytes [55]. Therefore, in this study, we have examined whether markers of insulin resistance and inflammation could mediate the association between RBP4 and T2DM. In the present set up, there was a significant reduction in mRNA expression of RBP4 in WAT in Q10 or PIO or Q10 + PIO treated rats. However, there was no significant

change in *RBP4* expression in Q10 + PIO as compared to monotherapy of Q10 or PIO.

Resistin gene is also a member of pro-inflammatory cytokine family [56,57] and responsible for the inhibition of adipogenesis with insulin resistance in human adipose tissues [58,59]. Other inflammatory cytokine like *TNF-α* and *IL-6* are also play a major role as mediators in the inflammatory pathway of adipocyte [60]. Hence, we measured the mRNA expression of *RETN*, *TNF-α* and *IL-6* in WAT in STZ-NA induced diabetic rats treated with Q10 or PIO or Q10 + PIO. In our finding, it was found that the mRNA expression level of *RETN*, *TNF-α* and *IL-6* were significantly up regulated in the STZ-NA induced diabetic rats, while there was a significant down regulation of these pro-inflammatory cytokines in the animals treated with Q10 or PIO or Q10 + PIO as compare to STZ-NA treated rats. However, combination of Q10 and PIO showed a more down regulation than Q10 or PIO administered alone. This observation shows that Q10 has a synergistic effect when co-administered with PIO suggesting a protective effect of Q10 in metabolic syndrome. This effect might be due to the antioxidant effect of Q10, thereby reducing the expression of inflammatory adipocytokines like *RETN*, *RBP-4*, *TNF-α* and *IL-6*.

6. Conclusion

We concluded that Q10 or PIO or Q10 + PIO exhibited a significant amelioration in the levels of FBG, serum insulin, HbA1c and biomarkers of oxidative stress along with mRNA expression of adipocytokines. However, Q10 + PIO combination is showed a better therapeutics effect on aforementioned parameters. However, this has to be proved in clinical setting.

Abbreviations

DM	Diabetes mellitus
T2DM	Type 2 diabetes mellitus
SOD	Superoxide dismutases
CAT	Catalase
GPx	Glutathione peroxidase
TNF-α	Tumor necrosis factor alpha
IL	Interleukin
IR	Insulin resistance
Q10	Coenzyme Q10
TK	Tyrosine Kinase
PI3K	Phosphoinositide 3-kinase
sRAGE	Receptor for advanced glycation end products
TZD	Thiazolidinediones
PPARγ	Peroxisome proliferator-activated receptor-γ
qRT-PCR	quantitative Real time PCR

Funding information

This work was supported by the Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara-391760, Gujarat, India (Ref. no. SVRC/ON/2015/15264) from Research cell, to Dr. Ramachandran Balaraman (PhD, FAMS) Principle Investigator.

Declaration of competing interest

There are no conflicts of interests.

Acknowledgement

We are thankful to Sumandeep Vidyapeeth Deemed to be University for the providing us the financial support to carry out present research work.

References

[1] B. Liu, Y. Xiang, Z. Liu, Z. Zhou, Past, present and future of latent autoimmune diabetes in adults, Diabetes Metab. Res. Rev. 36 (2020) e3205, , https://doi.org/10.1002/dmrr.3205.
[2] G.C. Weir, S. Bonner-Weir, Five of stages of evolving β-cell dysfunction during progression to diabetes, Diabetes. 53 (2004) S16, https://doi.org/10.2337/diabetes.53.suppl_3.S16.
[3] P. Zimmet, K.G. Alberti, D.J. Magliano, P.H. Bennett, Diabetes mellitus statistics on prevalence and mortality: facts and fallacies, Nat. Rev. Endocrinol. 12 (2016) 616–622, https://doi.org/10.1038/nrendo.2016.105.
[4] S.M. Fernandes, P.M. Cordeiro, M. Watanabe, C.D. da Fonseca, M. de F.F. Vattimo, The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats, Arch. Endocrinol. Metab. 60 (2016) 443–449, https://doi.org/10.1590/2359-3997000000188.
[5] F. Giacco, M. Brownlee, Oxidative stress and diabetic complications, Circ. Res. 107 (2010) 1058–1070, https://doi.org/10.1161/CIRCRESAHA.110.223545.
[6] L. Mahmoodnia, E. Aghadavod, S. Beigrezaei, M. Rafieian-Kopaei, An update on diabetic kidney disease, oxidative stress and antioxidant agents, J. Ren. Inj. Prev. 6 (2017) 153–157, https://doi.org/10.15171/jrip.2017.30.
[7] A.D. Attie, P.E. Scherer, Adipocyte metabolism and obesity, J. Lipid Res. 50 (2009) S395–S399, https://doi.org/10.1194/jlr.R800057-JLR200.
[8] M. Blüher, Adipose tissue dysfunction in obesity, Exp. Clin. Endocrinol. Diabetes 117 (2009) 241–250, https://doi.org/10.1055/s-0029-1192044.
[9] H. Gandhi, A. Upaganlawar, R. Balaraman, Adipocytokines: the pied pipers, J. Pharmacol. Pharmacother. 1 (2010) 9–17, https://doi.org/10.4103/0976-500X.64530.
[10] K. Maedler, P. Sergeev, F. Ris, J. Oberholzer, H.I. Joller-Jemelka, G.A. Spinas, N. Kaiser, P.A. Halban, M.Y. Donath, Glucose-induced β cell production of IL-1β contributes to glucotoxicity in human pancreatic islets, J. Clin. Invest. 110 (2002) 851–860, https://doi.org/10.1172/jci15318.
[11] A.B. Goldfine, V. Fonseca, S.E. Shoelson, Therapeutic approaches to target inflammation in type 2 diabetes, Clin. Chem. 57 (2011) 162–167, https://doi.org/10.1373/clinchem.2010.148833.
[12] R.B. Goldberg, Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications, J. Clin. Endocrinol. Metab. 94 (2009) 3171–3182, https://doi.org/10.1210/jc.2008-2534.
[13] R.A. Maheshwari, R. Balaraman, A.K. Sen, A.K. Seth, Effect of coenzyme Q10 alone and its combination with metformin on streptozotocin-nicotinamide-induced diabetic nephropathy in rats, Indian J. Pharmacol. 46 (2014) 627.
[14] G.D. Calderon, O.H. Juarez, G.E. Hernandez, S.M. Punzo, Z.D. De La Cruz, Oxidative stress and diabetic retinopathy: development and treatment, Eye 31 (2017) 1122–1130, https://doi.org/10.1038/eye.2017.64.
[15] B.J. Brabin, M. Hakimi, D. Pelletier, Dietary glutathione protects rats from diabetic nephropathy and neuropathy, J. Nutr. 131 (2001) 604S–615S, https://doi.org/10.1093/JN.
[16] J.W. Baynes, S.R. Thorpe, Role of oxidative stress in diabetic complications: a new perspective on an old paradigm, Diabetes. 48 (1999) 1–9, https://doi.org/10.2337/diabetes.48.1.1.
[17] D.M. Niedowicz, D.L. Daleke, The role of oxidative stress in diabetic complications, Cell Biochem. Biophys. 43 (2005) 289–330, https://doi.org/10.1385/CBB:43:2:289.
[18] M. Makni, M. Sefi, H. Fetoui, E.M. Garoui, N.K. Gargouri, T. Boudawara, N. Zeghal, Flax and pumpkin seeds mixture ameliorates diabetic nephropathy in rats, Food Chem. Toxicol. 48 (2010) 2407–2412, https://doi.org/10.1016/j.fct.2010.05.079.
[19] V.R. Punithavathi, P.S.M. Prince, R. Kumar, J. Selvakumari, Antihyperglycaemic, antilipid peroxidative and antioxidant effects of gallic acid on streptozotocin induced diabetic Wistar rats, Eur. J. Pharmacol. 650 (2011) 465–471, https://doi.org/10.1016/j.ejphar.2010.08.059.
[20] M. Kamei, T. Fujita, T. Kanbe, K. Sasaki, K. Oshiba, S. Otani, I. Matsui-Yuasa, S. Morisawa, The distribution and content of ubiquinone in foods, Int. J. Vitam. Nutr. Res. 56 (1986) 57–63.
[21] C. Schmelzer, I. Lindner, G. Rimbach, P. Niklowitz, T. Menke, F. Döring, Functions of coenzyme Q10 in inflammation and gene expression, BioFactors 32 (2008) 179–183, https://doi.org/10.1002/biof.5520320121.
[22] Y. Kohli, Y. Suto, T. Kodama, Effect of hypoxia on acetic acid ulcer of the stomach in rats with or without coenzyme Q10, Jpn. J. Exp. Med. 51 (1981) 105–108.
[23] G. Lenaz, R. Fato, G. Formiggini, M.L. Genova, The role of coenzyme Q in mitochondrial electron transport, Mitochondrion 7 (2007) S8–33, https://doi.org/10.1016/j.mito.2007.03.009.
[24] H. Yki-Järvinen, Thiazolidinediones, N. Engl. J. Med. 351 (2004) 1106–1118, https://doi.org/10.1056/NEJMra041001.
[25] P. Tontonoz, E. Hu, R.A. Graves, A.I. Budavari, B.M. Spiegelman, mPPARγ2: tissue-specific regulator of an adipocyte enhancer, Genes Dev. 8 (1994) 1224–1234, https://doi.org/10.1101/gad.8.10.1224.
[26] J. Mohapatra, M. Sharma, S. Singh, A. Chatterjee, P. Swain, R. Balaraman, P.R. Patel, M.R. Jain, Subtherapeutic dose of pioglitazone reduces expression of inflammatory adipokines in db/db mice, Pharmacology 84 (2009) 203–210, https://doi.org/10.1159/000235996.
[27] K.C. Choi, O.H. Ryu, K.W. Lee, H.Y. Kim, J.A. Seo, S.G. Kim, N.H. Kim, D.S. Choi, S.H. Baik, K.M. Choi, Effect of PPAR-α and -γ agonist on the expression of visfatin, adiponectin, and TNF-α in visceral fat of OLETF rats, Biochem. Biophys. Res. Commun. 336 (2005) 747–753, https://doi.org/10.1016/j.bbrc.2005.08.203.
[28] N. Maeda, M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida,

H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura, Y. Matsuzawa, PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein, Diabetes 50 (2001) 2094–2099, https://doi.org/10.2337/diabetes.50.9.2094.

[29] G.B.T. Moore, H. Chapman, J.C. Holder, C.A. Lister, V. Piercy, S.A. Smith, J.C. Clapham, Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice, Biochem. Biophys. Res. Commun. 286 (2001) 735–741, https://doi.org/10.1006/bbrc.2001.5460.

[30] G.R. Parmar, K. Pundarikakshudu, R. Balaraman, Antidiabetic and antihyperlipidemic activity of *Euphorbia thymifolia* L. extracts on streptozotocin-nicotinamide induced type 2 diabetic rats, J. Appl. Pharm. Sci. 7 (2017) 078–084, https://doi.org/10.7324/JAPS.2017.70811.

[31] I. Aeberli, R. Biebinger, R. Lehmann, D. L'Allemand, G.A. Spinas, M.B. Zimmermann, Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children, J. Clin. Endocrinol. Metab. 92 (2007) 4359–4365, https://doi.org/10.1210/jc.2007-0468.

[32] T.F. Slater, B.C. Sawyer, The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used, Biochem. J. 123 (1971) 805–814, https://doi.org/10.1042/bj1230805.

[33] H.P. Misra, I. Fridovich, Superoxide dismutase: a photochemical augmentation assay, Arch. Biochem. Biophys. 181 (1977) 308–312, https://doi.org/10.1016/0003-9861(77)90509-4.

[34] E.B. Hugo, S.P. Colowick, N.O. Kaplan, L. Packer, Oxidoreductases acting on groups other than CHOH: catalase, Methods Enzymol. 105 (1984) 121–125.

[35] M.S. Moron, J.W. Depierre, B. Mannervik, Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver, BBA - Gen. Subj. 582 (1979) 67–78, https://doi.org/10.1016/0304-4165(79)90289-7.

[36] J. Mohapatra, M. Sharma, S. Singh, G. Pandya, A. Chatterjee, R. Balaraman, P.R. Patel, M.R. Jain, Involvement of adipokines in rimonabant-mediated insulin sensitivity in ob/ob mice, J. Pharm. Pharmacol. 61 (2009) 1493–1498, https://doi.org/10.1211/jpp.61.11.0008.

[37] D.C. Rio, M. Ares, G.J. Hannon, T.W. Nilsen, Purification of RNA using TRIzol (TRI reagent), Cold Spring Harb Protoc 5 (2010), https://doi.org/10.1101/pdb.prot5439 pdb. prot5439.

[38] D.C. Rio, Denaturation and electrophoresis of RNA with formaldehyde, Cold Spring Harb Protoc (2015) (2015) 219–222, https://doi.org/10.1101/pdb.prot080994.

[39] Hassan Ahmadvand, Effects of coenzyme Q10 on hemoglobin A1C, serum urea and creatinine in alloxan-induced type 1 diabetic rats, Iran, J. Pharmacol. Ther. 11 (2006) 64–0 http://ijpt.iums.ac.ir/article-1-245-en.html (accessed July 14, 2020).

[40] F.M. Rauscher, R.A. Sanders, J.B. Watkins, Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats, J. Biochem. Mol. Toxicol. 15 (2001) 41–46, https://doi.org/10.1002/1099-0461(2001)15:1<41::AID-JBT5>3.0.CO;2-Z.

[41] M.L. Lukić, S. Stošić-Grujičić, A. Shahin, Effector mechanisms in low-dose streptozotocin-induced diabetes, Dev. Immunol. (1998), https://doi.org/10.1155/1998/92198.

[42] E. Jarald, S. Joshi, D. Jain, S. Edwin, Biochemical evaluation of the hypoglycemic effects of extract and fraction of *Cassia fistula* linn. in alloxan-induced diabetic rats, Indian J. Pharm. Sci. 75 (2013) 427–434, https://doi.org/10.4103/0250-474X.119823.

[43] J.L. Evans, I.D. Goldfine, B.A. Maddux, G.M. Grodsky, Are oxidative stress - activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes. 52 (2003) 1–8, https://doi.org/10.2337/diabetes.52.1.1.

[44] K. Haskins, B. Bradley, K. Powers, V. Fadok, S. Flores, X. Ling, S. Pugazhenthi, J. Reusch, J. Kench, Oxidative stress in type 1 diabetes, Ann. N. Y. Acad. Sci. 1005 (2003) 43–54, https://doi.org/10.1196/annals.1288.006.

[45] H. Ha, K.H. Kim, Pathogenesis of diabetic nephropathy: the role of oxidative stress and protein kinase C, Diabetes Res. Clin. Pract. 45 (1999) 147–151, https://doi.org/10.1016/S0168-8227(99)00044-3.

[46] F.M. Metwally, H.M. Rashad, H.H. Ahmed, A.A. Mahmoud, E.R. Abdol Raouf, A.M. Abdalla, Molecular mechanisms of the anti-obesity potential effect of *Moringa oleifera* in the experimental model, Asian Pac. J. Trop. Biomed. 7 (2017) 214–221, https://doi.org/10.1016/j.apjtb.2016.12.007.

[47] K.J. Catalano, D. Stefanovski, R.N. Bergman, Critical role of the mesenteric depot versus other intra-abdominal adipose depots in the development of insulin resistance in young rats, Diabetes 59 (2010) 1416–1423, https://doi.org/10.2337/db08-0675.

[48] C.J. De Souza, M. Eckhardt, K. Gagen, M. Dong, W. Chen, D. Laurent, B.F. Burkey, Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance, Diabetes 50 (2001) 1863–1871, https://doi.org/10.2337/diabetes.50.8.1863.

[49] A.B. King, D.U. Armstrong, Lipid response to pioglitazone in diabetic patients: clinical observations from a retrospective chart review, Diabetes Technol. Ther. 4 (2002) 145–151, https://doi.org/10.1089/15209150260007354.

[50] Y. Seino, H. Hirose, I. Saito, H. Itoh, High molecular weight multimer form of adiponectin as a useful marker to evaluate insulin resistance and metabolic syndrome in Japanese men, Metabolism 56 (2007) 1493–1499, https://doi.org/10.1016/j.metabol.2007.06.015.

[51] T.P. Combs, J.A. Wagner, J. Berger, T. Doebber, W.J. Wang, B.B. Zhang, M. Tanen, A.H. Berg, S. O'Rahilly, D.B. Savage, K. Chatterjee, S. Weiss, P.J. Larson, K.M. Gottesdiener, B.J. Gertz, M.J. Charron, P.E. Scherer, D.E. Moller, Induction of adipocyte complement-related protein of 30 kilodaltons by PPARγ agonists: a potential mechanism of insulin sensitization, Endocrinology 143 (2002) 998–1007, https://doi.org/10.1210/endo.143.3.8662.

[52] M.A. Statnick, L.S. Beavers, L.J. Conner, H. Corominola, D. Johnson, C.D. Hammond, R. Rafaeloff-Phail, T. Seng, T.M. Suter, J.P. Sluka, E. Ravussin, R.A. Gadski, J.F. Caro, Decreased expression of apM1 in omental and subcutaneous adipose tissue of humans with type 2 diabetes, Exp. Diabesity Res. 1 (2000) 81–88, https://doi.org/10.1155/edr.2000.81.

[53] T. Yamauchi, J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M.L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel, T. Kadowaki, The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity, Nat. Med. 7 (2001) 941–946, https://doi.org/10.1038/90984.

[54] Q. Yang, T.E. Graham, N. Mody, F. Preitner, O.D. Peroni, J.M. Zabolotny, K. Kotani, L. Quadro, B.B. Kahn, Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes, Nature 436 (2005) 356–362, https://doi.org/10.1038/nature03711.

[55] J. Norseen, T. Hosooka, A. Hammarstedt, M.M. Yore, S. Kant, P. Aryal, U.A. Kiernan, D.A. Phillips, H. Maruyama, B.J. Kraus, A. Usheva, R.J. Davis, U. Smith, B.B. Kahn, Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun N-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism, Mol. Cell. Biol. 32 (2012) 2010–2019, https://doi.org/10.1128/mcb.06193-11.

[56] M. Bokarewa, I. Nagaev, L. Dahlberg, U. Smith, A. Tarkowski, Resistin, an adipokine with potent proinflammatory properties, J. Immunol. 174 (2005) 5789–5795, https://doi.org/10.4049/jimmunol.174.9.5789.

[57] I.N. Holcomb, R.C. Kabakoff, B. Chan, T.W. Baker, A. Gurney, W. Henzel, C. Nelson, H.B. Lowman, B.D. Wright, N.J. Skelton, G.D. Frantz, D.B. Tumas, F.V. Peale, D.L. Shelton, C.C. Hébert, FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family, EMBO J. 19 (2000) 4046–4055, https://doi.org/10.1093/emboj/19.15.4046.

[58] S. Chung, K. LaPoint, K. Martinez, A. Kennedy, M.B. Sandberg, M.K. McIntosh, Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes, Endocrinology 147 (2006) 5340–5351, https://doi.org/10.1210/en.2006-0536.

[59] K.H. Kim, K. Lee, Y.S. Moon, H.S. Sul, A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation, J. Biol. Chem. 276 (2001) 11252–11256, https://doi.org/10.1074/jbc.C100028200.

[60] D. Lacasa, S. Taleb, M. Keophiphath, A. Miranville, K. Clement, Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes, Endocrinology 148 (2007) 868–877, https://doi.org/10.1210/en.2006-0687.