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Research Article

Antidiabetic Effect of Thymoquinone via Modulation of PPAR-γ, GLUT4, Hyperlipidemia and Antioxidant Status in Diabetic Rats

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Abstract

Background and Objective: The management of diabetic hyperglycemia, dyslipidemia and oxidative stress status are the key elements in the protection of diabetic complications. The present study was hypothesized to evaluate the antidiabetic, antihyperlipidemic and antioxidant effects of thymoquinone on streptozotocin-induced diabetic albino rats. **Materials and Methods:** The rats were divided into 2 categories; pre-treated and treated groups. Thymoquinone (50 mg kg^{-1} b.wt.) was intraperitoneally injected day after day for 2 weeks pre and 2 weeks after induction of diabetes for the pre-treated group and 2 weeks after induction of diabetes for treated group. **Results:** The current study revealed that thymoquinone administration caused amelioration in hyperglycemia, hypoinsulinemia, dyslipidemia, impaired antioxidant defense system and upregulation to PPAR-γ and GLUT4 genes expression as compared to the diabetic rats. **Conclusion:** The current results revealed the hypoglycemic, hypolipidemic and protective effects of thymoquinone against diabetes via potentiating insulin secretion and action, modulate PPAR-γ and GLUT4 genes expression and also by its potent antioxidant effect.

Key words: Thymoquinone, hyperglycemia, hyperlipidemia, diabetic complications, insulin secretion, antihyperlipidemic, PPAR-γ, GLUT4

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a widely spread epidemic disorder of the metabolism of carbohydrates and lipids, which caused a serious endocrine disorder that causes millions of deaths worldwide[1]. From 2012-2014, diabetes is estimated to have resulted in 1.5-4.9 million deaths each year[2]. For controlling diabetes, various treatments including diet, lifestyle changes, biochemical and herbal medicine in combination or alone have been used[3]. At the present time, traditional agents consist of those that enhance insulin secretion (i.e., sulfonylureas and glinides), those that enhance insulin sensitivity (i.e., metformin and the thiazolidinediones) and those that inhibit intestinal carbohydrate absorption (i.e., the alpha-glucosidase inhibitors) and the dipeptidyl peptidase-4 (DPP-4) inhibitors, which potentiate the activity of the incretin glucagon-like peptide1 and enhance glucose-dependent insulin secretion[4]. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge[5].

For thousands of year's natural products have played a very important role in health care and prevention of diseases. The ancient civilizations of the Chinese, Indians, North Africans and Egyptian provide written evidence for the use of natural sources for curing various diseases[6]. The WHO estimates that about 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Also, WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures[7]. There is a continuous need to develop new and better pharmaceuticals as alternatives for the management and treatment of diabetes. Therefore, in recent years, considerable attention has been directed towards the identification of plants with an antidiabetic ability that may be used for human consumption[8].

Nigella sativa or Black seed is one of the medicinal plants with anti-hyperglycemic and anti-hyperlipidemic characteristics[9]. *Nigella sativa* has many different chemical ingredients including Thymoquinone (TQ), the most prominent constituent of *Nigella sativa* seeds essential oil, which evidently proved its activity as hepatoprotective, anti-inflammatory, antioxidant and anticancer chemical, that provide support to consider this compound as an emerging drug[10]. Moreover, Badr *et al.*[11] suggested that the nutritional supplementation of diabetic dams with the natural antioxidant TQ during pregnancy and lactation protects their offspring from developing diabetic complications and preserves an efficient lymphocyte immune response later in life[11]. The TQ and proanthocyanidin may be clinically useful for protecting diabetic kidney against oxidative stress[12]. The treatment of TQ during pregnancy of diabetic mice inhibits the rate of embryo malformations by reducing the free radicals, in addition to increasing the size and maturation of embryos[13]. The actual hypoglycemic mechanism of TQ has not been discussed extensively and further investigations are needed to explore the different perspective mode of action. Thus, the present study aimed to investigate the effect of thymoquinone administration on hyperglycemia by modulation of PPAR-γ and GLUT4 genes expression, hyperlipidemic and antioxidant status in diabetic albino rats.

MATERIALS AND METHODS

Experimental materials: White male albino rats (*Rattus norvegicus*) weighing about 120-180 g were used as experimental animals in the present investigation. They were supplied from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC), Beni-Suef University, Egypt (BSU/FS/2015/12).

The TQ is a compound derived from the black seeds of a Middle eastern flower called *Nigella sativa*. The TQ was purchased from Sigma (Sigma-Aldrich Co., St. Louis, Missouri, USA). Oil-in-water nano-emulsion from thymoquinone (TQ) was prepared by homogenizing 5% of TQ with 95% aqueous phase (5% Tween-20 and 90% double distilled-water)[14], injected day after day in a dose of 50 mg kg^{-1} b.wt.

Methods

Induction of diabetes: Diabetes mellitus was experimentally induced in animals fasted for 16 h by intraperitoneal injection of 45 mg kg^{-1} b.wt., streptozotocin purchased from Sigma (Sigma-Aldrich Co., St. Louis, Missouri, USA) dissolved in citrate buffer[15], pH 4.5. Rats having serum glucose ranging from 200-300 mg dL^{-1} (mild diabetes), after 2 h of glucose intake, were included in the experiment, while the others were excluded.

Animals grouping: The animals (32 ones) were divided into 2 categories; the prophylactic groups (which administered TQ before diabetes induction) and the treated groups (which administered TQ after diabetes induction) and were divided into four groups (8 animals/group) from April, 2015 to July, 2016:

G1 : 1st group of normal animals were kept without treatments under the same laboratory condition and regarded as frank (normal control) group for the all groups

G2 : 2nd group was regarded as a diabetic control for all groups and kept after diabetes induction without treatments under the same laboratory condition for 2 weeks

G3 : 3rd group was injected the thymoquinone (TQ) nanoemulsion intraperitoneal 1 mL kg^{-1} b.wt., day after day for 2 weeks before streptozotocin injection and for another 2 weeks after diabetic induction (pre-treated group)

G4 : 4th group was injected the thymoquinone (TQ) nanoemulsion intraperitoneal 1 mL kg^{-1} b.wt., day after day for 2 weeks after diabetic induction (treated group)

By the end of the experimental period, normal, diabetic control, pre-treated and diabetic treated rats were sacrificed under mild diethyl ether anesthesia. The clear, non-heamolysed supernatant sera were quickly removed, divided into four portions for each individual animal and kept at -40°C for further analysis.

Determination of biochemical assays: The OGTT test was performed on normal, diabetic control, prophylactic and diabetic treated after treatment with TQ. Successive blood samples were then taken following the administration of 3 g glucose[16]/kg b.wt. The determination of glucose concentration was assayed by using the reagent kit obtained from Biochon (Germany) through Alkan medical agent. However, serum insulin was determined with radioimmuno-assay kit obtained from DPC (Diagnostic Products Corporation), Los Angeles, U.S.A Serum cholesterol, HDL-cholesterol, LDL-cholesterol, vLDL-cholesterol and triglycerides concentrations were estimated using reagent kit purchased from Reactivos Spinreact Company, Spain[17]. Moreover, the cardiovascular risk (CVR) and the antiatherogenic factor indices were calculated according to Ross[18]. On the other hand, malondialdehyde (MDA) (lipid peroxidation marker) concentration was determined in the liver homogenate according to the method of Yagi[19]. However, Nitric Oxide (NO) was determined indirectly by measuring the production of nitrites in the liver extract according to the method of the Griess diazotization reaction[20]. So, superoxide dismutase (SOD) was estimated according to the method of Kakkar *et al.*[21] while, glutathione peroxidase (GPX) was assessed by the method of Wendel[22]. Additionally, glutathione S-transferase (GST) and reduced glutathione (GSH) were assessed by the method of Habig *et al.*[23] and Moron *et al.*[24], respectively.

Detection of GLUT-4 and PPAR-γ gene expression by real time-PCR: Real-time quantitative polymerase chain reaction (qPCR) differs from regular PCR by including in the reaction fluorescent reporter molecules that increase proportionally with the increase of DNA amplification in thermocycler. There are two types of fluorescent chemistries for this purpose: Double strand DNA-binding dyes and fluorescently labeled sequence specific probe/primer. SYBR Green I dye and TaqMan® hydrolysis probe are the common examples for these two respectively. Total RNA was extracted from the visceral adipose tissue of each rats using TriFast™ reagent (PeQlab, Germany). The RNA was purified and spectrophotometrically quantified. The produced cDNA was amplified using *Go Taq* green master mix (Promega, USA) using the following sets of primers; 5-ACATACCTGACAGGG CAAGG-3 (forward) and 5-CGCCCTTAGTTGGTCAGAAG-3 (reverse) for glucose transporter type 4 (GLUT4) and 5-GCCCTT TGGTGACTTTATGGA F-3 (forward) and 5-GCAGCAGGTTGTCTT GGATG-3 (reverse) for PPAR-γ. The PCR was performed using green master mix (Promega, USA) and T100™ thermal cycler (Bio-Rad Laboratories, USA) under the following conditions: initial denaturation at 95°C for 5 min, 35 cycles set at 94°C (1 min) for denaturation, 55°C (1 min) for annealing and 72°C (1 min) for extension and finally at 72°C (5 min) to complete the extension reaction. The PCR products were subjected to electrophoresis on 1.5% agarose gels containing ethidium bromide. Images from electrophoresed gels were captured by a camera in a computer assisted gel documentation system. Relative band intensities of each sample were calculated after being normalized with the band intensity of β-actin using phoretix 1-D densitometry software v.11 (Total Lab Ltd., UK) and values presented as mRNA relative (%) to control.

Statistical analysis of results: The Statistical Package for the Social Sciences (IBM SPSS for WINDOWS 7, version 22; SPSS Inc., Chicago) was used for the statistical analysis. Comparative analysis was conducted by using the general linear models procedure (IBMSPSS). The results were expressed as mean±standard deviation (SD) and values of $p<0.05$ were considered statistically significant at $p<0.05$.

RESULTS

Effect of TQ on levels of glucose, insulin, PPAR-γ and GLUT4 genes expression: Concerning the current results, both thymoquinone pretreated and treated groups had significant ($p<0.05$) decrease in fasting serum glucose levels when

Table 1: Fasting Blood Glucose (FBS), insulin level, PPAR-γ and GLUT-4 genes expression of normal, diabetic control, thymoquinone pretreated and treated groups

	Groups				
Parameters	Nor-C	Diab-C	TQ-P	TQ-T	F-value
FBS (mg dL^{-1})	79.61±3.95^{e}	149.67±3.20^{a}	98.07±2.56^{c}	89.17±2.61^{d}	p<0.05
Insulin (uIU mL^{-1})	16.13±4.47^{e}	4.64±0.92^{a}	9.05±0.98^{c}	8.05±1.08^{bc}	
PPAR-γ	1.02±0.01^{c}	0.31±0.13^{a}	0.52±0.18^{ab}	0.54±0.18^{b}	
GLUT-4	1.05±0.04^{c}	0.21±0.07^{a}	0.61±0.21^{b}	0.60±0.18^{b}	

Number of animals in each group was seven, data are expressed as Mean±SD, Means which shared the same superscript symbol(s) are non-significantly different (p>0.05) while others significantly different (p<0.05), Nor-C: Normal control, Diab-C: Diabetic Control, TQ-P: Thymoquinone pretreated, TQ-T: Thymoquinone treated

Table 2: Lipid profile, serum total cholesterol, triglycerides, high-density-lipoprotein (HDL), low-density-lipoprotein (LDL), very-low-density-lipoprotein (vLDL), cardiovascular risk factors (1 and 2) and antiatherogenic factor of normal, diabetic control, thymoquinone pretreated and treated groups

	Groups				
Parameters	Nor-C	Diab-C	TQ-P	TQ-T	F-value
Cholesterol (mg dL^{-1})	163.71±16.83^{c}	231.14±10.7^{a}	185.14±7.7^{b}	163.40±9.85^{c}	p<0.05
Triglycerides (mg dL^{-1})	77.81±5.90^{c}	126.14±8.63^{a}	95.62±15.0^{b}	92.50±3.62^{b}	
HDL-C (mg dL^{-1})	83.00±5.39^{d}	35.00±3.56^{a}	54.00±4.4^{c}	56.64±3.06^{c}	
LDL-C (mg dL^{-1})	65.15±10.64^{d}	170.91±7.69^{a}	112.02±2.6^{b}	88.29±6.24^{c}	
vLDL-C (mg dL^{-1})	15.56±1.18^{c}	25.23±1.73^{a}	19.12±3.1^{b}	18.5±0.72^{b}	
C risk factor 1	1.97±0.09^{e}	6.64±0.49^{a}	3.44±0.2^{c}	2.89±0.05^{d}	
C risk factor 2	0.78±0.08^{e}	4.92±0.46^{a}	2.09±0.2^{c}	1.56±0.05^{d}	
Antiatherogenic factor	103.98±9.81^{e}	17.85±1.71^{a}	41.15±2.6^{c}	53.09±1.38^{d}	

Number of animals in each group was seven, data are expressed as Mean±SD, Means which shared the same superscript symbol(s) are non-significantly different (p>0.05) while others significantly different (p<0.05)

Table 3: Oxidation biomarkers, lipid peroxidation (MDA), Nitric Oxide (NO), superoxide dismutase (SOD), glutathione peroxidase (GPx) glutathione transferase (GST) and glutathione reduced form (GSH) of normal and diabetic control, thymoquinone pretreated and treated groups

	Groups				
Parameters	Nor-C	Diab-C	TQ-P	TQ-T	F-value
MDA (U/100 mg T)	33.37±3.04^{c}	62.41±3.64^{a}	39.53±5.28^{c}	35.99±2.49^{cd}	p<0.05
NO (U L^{-1})	4.42±0.48^{d}	12.27±2.16^{a}	7.00±1.01^{c}	6.99±0.74^{c}	
SOD (U GT^{-1})	811.78±63.2^{d}	263.06±34.33^{a}	785.91±29.11^{d}	619.51±46.68^{c}	
GPx (U GT^{-1})	120.28±9.23^{d}	66.24±4.36^{a}	79.23±1.88^{b}	77.15±4.91^{b}	
GST (U GT^{-1})	1318.6±128.6^{e}	419.20±19.26^{a}	954.89±42.73^{c}	1121.89±62.60^{d}	
GSH (U GT^{-1})	1144.99±72.41^{c}	26.08±23.23^{a}	452.00±19.2^{a}	884.35±52.48^{b}	

Number of animals in each group was seven, data are expressed as Mean±SD, Means which shared the same superscript symbol(s) are non-significantly different (p>0.05) while others significantly different (p<0.05)

compared to diabetic control. However, both thymoquinone groups showed a significant (p<0.05) increase in fasting serum insulin levels when compared to diabetic control. Also, thymoquinone groups showed significantly (p<0.05) increases in PPAR-γ and GLUT-4 genes expression as compared to diabetic control (Table 1).

Effect of treatments on lipid profile: Hypercholesterolemia characterizing diabetic rats was significantly (p<0.05) decreased in both TQ pretreated and treated groups when compared to the diabetic control ones. Otherwise, TQ pretreated and treated groups induced significant (p<0.05) decrease in serum triglycerides level as compared to untreated diabetic control group. The decrease in serum HDL of diabetic group improved significantly (p<0.05) in both TQ groups, while TQ administration exhibited a significant decrease in LDL level when compared to diabetic control. Furthermore, TQ groups revealed a significant decrease in elevated serum vLDL when compared to the diabetic control group at the end of the experiment. Otherwise, the antiatherogenic factor showed a decreased in diabetic rats, however this decrease improved significantly (p<0.05) in the TQ pretreated and treated experimental groups when compared to diabetic control (Table 2).

Liver oxidative stress and antioxidant defense system: The diabetic animals revealed a significant (p<0.05) increase in lipid peroxidation (MDA), while TQ groups exhibited a significant (p<0.05) decrease in MDA level as compared to diabetic control. However, TQ exhibited a significant decrease (p<0.05) in elevated Nitric Oxide (NO) concentration as compared to diabetic control (Table 3). Moreover, TQ pretreated and treated groups exhibited significant (p<0.05) increases in liver SOD, GPX and GST activities when compared

to diabetic control. Otherwise, TQ treated groups had a significant ($p<0.05$) increase in reduced glutathione (GSH) level as compared to diabetic control group (Table 3).

DISCUSSION

Streptozotocin is a selective β-cell genotoxicant and when administrated in a single high dose it induces a rapid onset of diabetes[25]. In the present study, thymoquinone (TQ) showed a significant amelioration in insulin levels to obtain glycaemic control, which leads to a significant improvement of the oral glucose tolerance test (OGTT). So, Abdel-Moneim *et al.*[9] demonstrated that *Nigella sativa* (NS) seed decreased Fasting Blood Sugar (FBS); 2 h postprandially glucose (PPBS) and insulin resistance without any renal or hepatic side effects in patients with type 2 diabetes. In accordance with current result, Kanter *et al.*[26] reported that the administration of thymoquinone orally to STZ-diabetic rats significantly increased insulin levels. In addition, the anti-diabetic action of thymoquinone is at least partially mediated through a decrease in hepatic gluconeogenesis[27].

Diabetes is associated with profound alterations in the lipid profile and each of the lipid abnormalities is associated with an increased risk of coronary heart disease[28]. In the current study, TQ administration decreased significantly the elevated levels of TC, TG, LDL-C and vLDL-C as compared with the diabetic group, with a significant increase in HDL-C level. These results were supported by the finding of Atta *et al.*[29] who concluded that TQ administration improves glucose homeostasis and lipid profiles in STZ-diabetic rats.

The results of the current study showed that there were significant decreases in liver PPAR-γ and GLUT4 expression genes. The study also, showed that both TQ pretreated and treated groups showed increased levels of PPAR-γ and GLUT4 mRNA expressions significantly as compared with the diabetic group. Recent evidence has demonstrated a role for a member of the nuclear hormone receptor superfamily of proteins in the etiology of type 2 diabetes[30]. The PPAR-γ is essential for adipocyte differentiation and hypertrophy and mediates the activity of the insulin-sensitizing thiazolidinediones (TZDs)[31]. However, when diabetic rats are treated with PPAR-γ agonists, PEPCK and glucose-6-phosphatase expressions are decreased and lipogenic gene expressions are increased, suggesting that PPAR-γ agonists decrease gluconeogenesis and increase adipogenesis and glycolysis[32]. Moreover, in diabetic rats treated with PPAR-γ agonists, it induced a decrease in free fatty acid levels precedes the decrease of glucose and triglyceride levels, suggesting that a decrease in free fatty acid levels may be important for the insulin-sensitizing action of PPAR-γ agonists[33]. Moreover, activation of PPAR-γ induces the differentiation of preadipocytes into adipocytes and stimulates triglyceride storage. The PPAR-γ, by increasing triglyceride storage and improving insulin sensitivity, is rather a "well-fed-lipid storing-glucose utilizing" regulator[34]. On the other hand, upregulation of PPAR-γ mRNA expression exhibited marked antidiabetic action in diabetic rats treated with gallic acid and p-coumaric acid[35].

Glucose transporter type 4 (GLUT4) deficiency resulted in decreased levels of lactate and FFAs in both the fasting and fed states and of β-hydroxybutyrate in the fasting state. These changes are the opposite of those seen with GLUT4 overproduction and also the opposite of those seen in the diabetic phenotype. Because GLUT4 is dysregulated in diabetes and obesity, it was expected that genetic ablation of GLUT4 would result in abnormal glucose homeostasis[36]. Rats fed TQ showed an increase in GLUT4 protein content, compared to the respective control groups. The TQ influences GLUT4 through the AMPK pathway. Activation of AMPK results in translocation of GLUT4 to the plasma membrane which mobilizes glucose into the cell[37].

Concerning current data, the pretreated and treated groups of TQ reduced significantly the liver tissue levels of malondialdehyde (MDA) and nitric oxide (NO) as compared with the diabetic group. However, pretreated and treated groups of TQ ameliorated significantly the reduced liver tissue levels of superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione S-transferase (GST) as compared with the diabetic group. The TQ administered to diabetic rats leads to a significant increase in glutathione reduced form (GSH) and superoxide dismutase (SOD) as compared to diabetic control rats[38]. The TQ supplementation also normalized liver reduced glutathione (GSH) and decreased the levels of MDA activity in the liver[39]. Pretreatment of Wistar rats with TQ and 1,2-dimethylhydrazine (DMH) for 10 weeks prevented the depletion of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase (SOD) in red blood cells and maintained a similar value as the control group[40]. The TQ exerts a protective action on pancreatic beta cell function and overcomes oxidative stress through its antioxidant properties[41]. *Nigella sativa* and thymoquinone may prove clinically useful in the treatment of diabetics and in the protection of β-cells against oxidative stress[42]. Referring to TQ as an antioxidant, the antioxidant effect of TQ can influence the antidiabetic mechanism through it can protect against STZ-induced beta cell destruction.

CONCLUSION

Thymoquinone administration showed a protective and ameliorative effect against hyperglycemia via increasing the insulin secretion and action, activation of PPARγ and GLUT4 genes expression and ameliorating the hyperlipidemia as well as the oxidative stress status.

SIGNIFICANCE STATEMENT

This study provided valuable information and guidance for using thymoquinone in the treatment of diabetes. The result revealed the beneficial role of thymoquinone on increase PPAR-γ and GLUT4 genes expression which contributes to increasing insulin sensitivity and the hypoglycemic action as well as attenuating the hyperlipidemia and antioxidant defenses. Also, our study will help the researchers to clear the critical antidiabetic mechanisms of thymoquinone administration that may help researchers to target it as a prospective oral hypoglycemic drug.

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