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Review Article

Thymoquinone, a Panacea for Diabetic Complications - An Overview

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Abstract

Diabetes mellitus, a chronic metabolic disorder has became an epidemic worldwide. Genetic predisposition, life style modifications with a paradigm shift in food habits are involved in the pathogenesis of diabetes mellitus. Hyperglycemia and the associated biochemical alterations causes various micro and macrovascular complications. Naturally occurring phytochemicals have find immense applications in the management of diabetes mellitus. Novel agents that can act on multiple targets are beneficial in curtailing the complications of diabetes mellitus. Thymoquinone (TQ), the active principle in the volatile oil of *Nigella sativa* seeds possess diverse pharmacological activities. Nowadays combination therapy is evolving as a means for optimizing glycemic control in diabetes mellitus. Additionally, drugs to prevent or delay the secondary complications are also required increasing the burden on diabetic individuals. As a single therapeutic agent thymoquinone optimises glycemic control and also curtails secondary complications signifying its uniqueness in the management of type 2 diabetes mellitus. In this context, this review highlights the influence of TQ on diabetes associated cardiovascular, neuronal, renal and reproductive defects and suggest its wide application as a potential therapeutic agent for diabetes and its complications.

Keywords: *Thymoquinone; Diabetic Complications*

Background

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia resulting from defective insulin secretion and action or both. The incidence of diabetes mellitus is rising at an alarming rate worldwide. Persistent hyperglycemia leads to micro and macrovascular complications, thereby increasing morbidity and mortality. To overcome diabetes associated complications and to improve pancreatic β-cell function, a variety of approaches that targets diverse molecular pathways are investigated. The search for lead compounds from natural sources are always promising as they possess diverse pharmacological activities with minimal side effects.

Spices have been closely connected to medicine since early human history. Among them *Nigella sativa*, a spice also known as "black seed" or "black cumin" has been found to rank high among the antidiabetic plants most recommended by traditional practitioners [1]. It is very popular in various traditional systems of medicine like Unani, Ayurveda and Siddha. Historical references to these seeds are found in some of the oldest religious and medical texts, where it has been stated as 'Melanthion' by Hippocrates and Dioscorides, and as 'Curative black cumin' in Bible and as 'Blessed seeds' in Quran [2]. *Nigella sativa* is an annual flowering plant of the Ranunculacea family native to southwest Asia. It grows to 20 - 30 cm tall with finely divided leaves. The fruit is a large inflated capsule composed of 3 - 7 united follicles, each containing numerous seeds. The seeds are a good source of essential nutrients. They have a pungent bitter taste and are used for edible and medicinal purposes. The seeds were shown to contain fixed oil (> 30% w/w) and essential oil (0.40 - 0.45%). Thymoquinone (TQ) (Figure 1) the principal active ingredient is present approximately 28 - 57 % in the essential oil of *Nigella sativa* seeds [3].

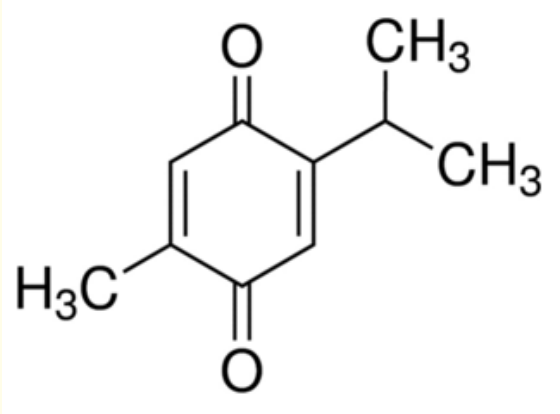

Figure 1: *Thymoquinone.*

In the diabetic state, persistent hyperglycemia leads to oxidative stress and causes damages to cellular macromolecules. In addition, dysfunction of pancreatic β-cell leads to insulinopenia and brings profound alterations in metabolism causing disturbances in glucose homeostasis. Previously, we studied the effect of TQ on the activities of key enzymes of carbohydrate metabolism and in the levels of endogenous antioxidants in diabetic rats. TQ improved antioxidant status and ameliorated the activities of carbohydrate metabolic enzymes through its insulinotrophic and antioxidative properties [4]. A large number of studies have revealed that TQ affects numerous molecular and signaling pathways in many degenerative diseases [5]. In this context, the objective of this study is to highlight the therapeutic role of TQ on pathways related to inflammation, apoptosis, and oxidative stress involved in cardiovascular, reproductive, renal and neuropathic complications of diabetes mellitus.

Evidence acquisition

Database using Google scholar and PubMed search engines were utilized for the literature search. The literature search was restricted to English language only. The Boolean operators AND/OR was used between the key words to retrieve maximum literature and the following key words were used in the above mentioned database. The key words used are TQ diabetic kidney, TQ diabetic neuropathy, TQ diabetic heart and TQ diabetic reproductive function.

Price and availability of TQ

S. No	Name of the Company	Quantity	Price (₹)
1	Cayman Chemical	500 mg	1733.66
2	Cayman Chemical	1g	2288.43
3	Cayman Chemical	5g	7836.15
4	Sigma-Aldrich	5g	10055.24
5	Sigma-Aldrich	1g	3210.74
6	Sigma-Aldrich	100 mg	3515.87
7	TCI Chemical	1g	4784.91
8	TCI Chemical	5g	17752.70

Table

Role of TQ in diabetes associated cardiovascular complications

Cardiovascular complications are the major cause of morbidity and mortality in patients with diabetes. The American Heart Association considers diabetes as one of the seven major controllable risk facts for CVD. Statistical data shows a strong correlation between cardiovascular disease (CVD) and diabetes [6]. The increased vulnerability of diabetic subjects to cardiovascular complications highlights the need for developing novel therapeutic agents.

To ascertain the role of TQ on cardiovascular function, Liu., *et al*. [7], focussed on oxidative stress (OST), inflammatory, apoptotic markers and on PI3K/Akt signaling in the cardiac tissue of streptozotocin (STZ) induced diabetic rats. In their study, male albino wistar rats were made diabetic by a single administration of STZ (60 mg/kg b.w) and were treated with TQ at a dose of 50 mg/kg b.w for 30 days. TQ administration improved insulin and decreased glucose levels suggesting its antidiabetic potential. The heart rate was found to be decreased with no significant changes in blood pressure. However, the ratio of heart, liver, lung to bodyweight were not altered in all the experimental groups. TQ decreased OST, as it is evidenced by an increase in the activity of superoxide dismutase, the primary enzyme involved in the dismutation of superoxide anion to hydrogen peroxide. This resulted in significant reduction of malondialdehyde (MDA), a lipid peroxidation product. Under physiological conditions, endothelial nitric oxide synthase (eNOS) catalyses the formation of nitric oxide (NO) that exerts beneficial effects on vasculature. However, hyperglycemia induced upregulation of eNOS expression resulted in the generation of superoxide anion which impairs endothelial function. TQ administration significantly decreased the expression of eNOS thereby decreasing the production of superoxide anion and confirmed its potent antioxidant effects.

Infiltration of inflammatory proteins induces apoptosis in the cardiac tissue of diabetic rats. In the diabetic state, elevated cyclooxygenase-2 (COX-2) expression is associated with altered prostanoid profile resulting in inflammation. In their study, TQ significantly decreased the expression of COX-2 and the serum levels of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) in diabetic treated rats. Also decreased activity of caspase 3 was observed in the cardiac tissue of TQ treated diabetic rats confirming its anti-inflammatory and anti apoptotic properties. Several studies documented that downregulation of Akt signaling is implicated in the pathogenesis of atherosclerosis, cardiac hypertrophy and vascular remodeling [8]. Akt expression was significantly upregulated in TQ treated diabetic rats suggesting an improvement in PI3K/Akt signaling pathway.

Influence of TQ on diabetic neuropathy

Among the chronic microvascular complications of diabetes, diabetic peripheral neuropathy (DPN) is responsible for 50 - 75% of non-traumatic lower limb amputations [9]. It is characterized by pathologic changes in peripheral nerves resulting from activation of protein kinase C, oxidative and inflammatory stress, advanced glycation end-product formation and alterations in polyol pathway [10]. Schwann cells are the principal glia of the peripheral nervous system which forms protective sheath around peripheral nerves. These cells also insulate axons from the surrounding endoneurial microenvironment by elaborating the segmental myelin sheaths of myelinated fibres. Responses of Schwann cells to diabetes-induced hyperglycemia are central to the pathogenesis of diabetic neuropathy.

In this context, Chen., *et al.* [11], cultured RSC96 cells (Schwann cells) at different glucose concentrations (50, 100, 150, and 200 mM) and studied the dose dependent action of TQ (5, 20, and 80 μM) on the proliferation and apoptosis of these cells. Glucose induced inhibition of proliferation of schwann cells were alleviated by TQ at the dose of 20 μM when compared to other two doses. Similarly, exposure of cells to various glucose concentrations resulted in apoptosis. The apoptotic rate significantly decreased on treatment with TQ at the dose of 20 μM than the other two doses, suggesting its antiapoptotic properties. In addition, the expression of COX-2, a key enzyme in inflammatory pathway was upregulated in schwann cells exposed to high glucose concentration and lipopolysaccharide (LPS). TQ at the doses of 5 and 20 μM downregulated the expression of COX-2 suggesting its anti-inflammatory potential. This effect of TQ was significant at the dose of 20 μM and is comparable with the selective COX-2 inhibitor NS-398. To further study the *in vivo* effects of TQ on the electrophysiological and morphological changes of the sciatic nerve, adult male wistar rats were fed high fat and high sugar

diet for 6 wks and injected intraperitoneally (i.p) with a single dose of STZ (30 mg/kg b.w). Diabetic rats were treated with TQ at 2 and 5 mg/kg b.w for 6 wks. The nerve conduction velocity (NCV) and sensory nerve conduction velocity (SNCV) were improved in DPN rats and the effect of TQ was comparable with vitamin $B_{12.}$ Morphological alterations in the sciatic nerve and the ultrastructural changes of myelin sheath were observed by haematoxylin and eosin staining and by transmission electron microscopy respectively. TQ attenuated the changes both in the sciatic nerve and in the myelin sheath. The expression pattern of COX-2, Caspase-3 in the sciatic nerve and the levels of IL-1β and 6 in the plasma were downregulated on treatment with various doses of TQ to DPN rats. From these findings, they concluded that TQ exerted a protective effect on DPN through its anti-inflammatory property.

Hamdy and Taha in 2009 [12], studied the neuroprotective effect of *Nigella sativa* oil (NSO) and TQ in the brain tissue of STZ induced diabetic rats. Adult male albino wistar rats were made diabetic by a single i.p injection of STZ at a dose of 60 mg/kg b.w. Diabetic rats were administered orally with NSO and TQ at the doses of 1 ml/kg and 10 mg/kg b.w respectively for a period of 14 days. The activities of catalase, glutathione-S-transferase (GST) and the levels of reduced glutathione (GSH) in the brain tissue of diabetic rats were improved significantly on treatment with NSO and TQ. The levels of norepinephrine, dopamine were increased with a significant fall in serotonin levels in the brain homogenate of NSO and TQ treated diabetic rats. Similarly, the levels of NO and MDA were decreased in diabetic treated rats. These results concluded that TQ and NSO exerted antioxidant properties and protected brain tissue in diabetic rats.

In 2008, Kanter [13] studied the beneficial effects of *Nigella sativa* (NS) and TQ on the histopathological changes of sciatic nerves in STZ (50 mg/kg b.w) induced diabetic rats.

To one group of diabetic rats, a dose of 400 mg/kg b.w NS and to the other 50mg/kg b.w TQ were given orally for a period of 12 wks. Immunohistochemical analysis of pancreatic tissue revealed an improvement in the immunoreactivity to insulin in both NS and TQ treated rats. Also, an increase in insulin levels with decreased plasma glucose and improved body weight were observed in diabetic treated rats. The myelinated fibre area and density of sciatic nerves were assessed by histological analysis. Similarly, the degenerative changes in the schwann cells and sciatic nerves were analysed by ultrastructural analysis. The structural features of axon, myelin and the schwann cells were improved in both NS and TQ treated diabetic rats confirming their neuroprotective effects.

The impact of TQ on reproductive function in diabetic subjects

Diabetes mellitus severely impairs reproductive function both in males and females. Recent data shows that there is a steady increase in diabetes mellitus among young individuals which is of great concern as they suffer infertility during their reproductive age. Erectile dysfunction and lower sex drive resulting from decreased testicular weight, abnormal spermatogenesis, sperm deformities with reduced ejaculate volume while menstrual cycle abnormalities like oligomenorrhea and secondary amenorrhea are the common findings in diabetic men and women. Several studies focused the molecular mechanisms responsible for the alterations in male reproductive potential induced by DM. These include changes in aromatase activity, alterations in sperm parameters and oxidative stress [14].

The physiological role of estrogens in the development and maintenance of testicular function is well established. Though estrogens are considered as female sex hormones it influences testicular and epididymal function and exerts multiple levels of control in spermatogenesis. The local production of estrogen from testosterone in mammalian testis is catalysed by aromatase, an endoplasmic reticulum enzyme complex. Epidemiological studies have shown that alterations in sperm morphology, diminution in sperm number and motility have been attributed to decreased aromatase gene expression. Insulin regulates the activity of aromatase and in diabetes the expression level of this enzyme is significantly decreased in testis [15]. Thus, aromatase has become an important target molecule to treat sexual dysfunction in diabetes mellitus.

Oxidative stress has been implicated in the pathogenesis of reproductive dysfunction in both type1 and type 2 diabetic subjects. The antioxidant defence capacity of the different testicular cell varies and suggests that these cells are sensitive to the deleterious effects of free radicals. The membranes of spermatozoa are susceptible to free radical attack because of their high level of polyunsaturated fatty

acids and also they have a reduced capability to repair DNA damage. Studies have shown that men suffering from diabetes have sperms with greater DNA fragmentation and an increase in advanced glycation end products and their receptors (RAGE) leading to deterioration of sperm quality.

In this context, the role of thymoquinone in ameliorating the defects in reproductive function of diabetic rats was studied by Atta., *et al.* in 2017 [16]. In their study, they investigated the molecular mechanisms by which TQ prevents diabetes induced testicular damage in rats. To experimentally induced diabetic rats (STZ; 65 mg/kg/b.w., i.p) they administered TQ orally at a dose of 50 mg/kg b.w for 12 wks. TQ administration increased relative weight of testicles, prostate, seminal vesicles, epididymis and improved sperm parameters such as sperm concentration, motility and viability in diabetic rats. An upregulation of aromatase with downregulation of iNOS (inducible nitric oxide synthase) and NF-kB expressions were observed in the testicular tissue of diabetic treated rats. Similarly, improvement in testicular reduced glutathione (GSH) level with increased SOD activities and a decrease in NO level were observed in the testicular tissue of TQ treated diabetic rats. Histological analysis of the test is revealed that TQ restored the architecture of testis and improved spermatogenesis in diabetic rats. From these findings they concluded that TQ is an excellent protective agent to improve fertility in diabetic subjects.

Influence of thymoquinone on renal function

Diabetic nephropathy (DN), is a leading cause of morbidity and mortality in diabetic subjects. The disease is characterized by thickening of glomerular basement membrane, expansion of mesangial cells, loss of podocytes with functional abnormalities leading to end stage renal diseases (ESRD) [17]. DN and ESRD continue to be a major challenge to health care systems and are imposing enormous mental, physical and social burdens. Though the aetiology is multifactorial, chronic hyperglycemia, oxidative stress and inflammation plays a decisive role in the onset and progression of DN [18].

Al-Trad., *et al.* in 2016 [19], studied the molecular mechanisms by which NSO and TQ exerted their actions in the renal tissue of diabetic rats. Their study focussed on albuminuria, podocyte injury, and the complex systems that controls the extracellular matrix accumulation and angiogenisis in adult female wistar rats administered with STZ (55 mg/kg b.w). They treated rats with 2 mL/kg NSO and 50 mg/kg TQ via oral gavage once a day for 10 wks. Podocytes are responsible for the maintenance of ultrastructure of glomerulus and their damage was assessed by measuring podocin levels by qRT-PCR and by immunostaining techniques. Injury or loss of podocytes results in marked proteinuria, a characteristic feature of renal damage commonly seen in diabetic subjects. In this study, the expression and distribution of podocin was completely recovered in TQ treated group and partially in NSO treated group suggesting their renoprotective effects. Further, the over expression of collagen IV, transforming growth factor-β1 (TGF-β1) and vascular endothelial growth factor-A (VEGF-A) were downregulated in the renal tissues of diabetic treated rats. From these findings, they concluded that NSO and TQ preserved podocyte function and suppressed ECM protein accumulation and angiogenesis in the renal tissue of diabetic rats.

Sayed., *et al.* [20] studied the mechanism by which TQ, Proanthocyanidin (PA) and their combination improved renal injury in STZ diabetic rats. To STZ (65 mg/kg b.w) induced male albino diabetic rats, they orally administered TQ (50 mg/kg b.w), PA (250mg/kg b.w) and their combination (50+250 mg/kg b.w), for 12 wks. Administration of these phytochemicals improved endogenous antioxidants such as GSH, SOD activity and prevented the elevation of urea, creatinine, nitric oxide, malondialdehyde and IL-6 in diabetic treated rats. They concluded that TQ and PA curtailed OST and attenuated DN in diabetic rats through their antioxidant properties.

Omran in 2014 [21] highlighted the possible effects of TQ on the morphological features of glomeruli and tubules. To male albino rats STZ at a dose of 60 mg/kg b.w was administered and were treated with TQ (50 mg/kg b.w) for a period of 8 wks. The progression of nephropathy in diabetic rats was assessed by measuring the expression of epithelial mesenchymal transformation (EMT) markers such as fibroblast- specific protein (Fsp1), desmin, matrix metalloproteinases (MMP-17) and Zonula occludens (ZO-1). Results confirmed a moderate expression of ZO-1 with significant decrease in the expression of Fsp-1, desmin and MMP-17 in diabetic treated

rats. Histochemical analysis revealed improvement in glomeruli, renal tubules and blood vessels in TQ treated rats. Similarly, the levels of creatinine, blood urea nitrogen (BUN), cystatin C were decreased in TQ administered rats. The study concluded that TQ exhibited protective effects in diabetic nephropathy.

In 2009, Kanter [22] investigated the potential of TQ in STZ induced nephropathy in experimental diabetic rats. Adult male wistar rats were made diabetic by a single i.p injection of STZ at a dose of 50 mg/kg b.w. Diabetic rats were given orally TQ at a dose of 50 mg/kg b.w for a period of 12 wks. TQ administration increased insulin and decreased serum glucose levels. Histological studies on the renal tissues revealed abnormal thickening of capsular wall, alterations in the basement membranes of glomerulus and tubules, dilatation of tubules in diabetic rats which were significantly reduced in TQ treated rats. Similarly, the increased expression of iNOS in the mesangium, podocytes and in the capillary loop of glomerulus were downregulated on treatment with TQ in diabetic rats. Further electron microscopic studies showed that TQ administration improved the morphological alterations in renal tissue of diabetic rats. From the above findings they concluded that TQ is useful in the treatment of diabetic nephropathy.

As TQ acts on multiple molecular targets it has been recommended as an anti-oxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial, anti-tumor and anti-diabetic agent. In addition to its multiple therapeutic properties, TQ finds immense applications in various industries. Many health care and cosmeceutical products containing TQ as an ingredient are currently being manufactured. These include, soft gelatin capsule as natural antioxidant and immunobooster, soaps as body cleansing agent, oils for complete hair care, creams to improve skin texture and pigmentation and shampoos for strong and shiny hair, thus highlighting its wide industrial application.

Conclusion

Herbs and their active constituents are used both as a prophylactic and therapeutic agents. This meta data on TQ reveals its therapeutic potential against the secondary complications of diabetes mellitus. As TQ modulates enzyme activities, inflammatory, antioxidant, apoptotic markers and transcription factors, it can be incorporated in the therapeutic regimen to bring health benefits in several degenerative diseases. However, to quantify the effective therapeutic dose, efficacy and safety, clinical trials are needed for translation of this drug to human subjects.

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