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An overview on cardioprotective and anti-diabetic effects of thymoquinone

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ABSTRACT

Thymoquinone (TQ), one of the active components of *Nigella sativa* exhibited to have many biological effects. Several beneficial effects of TQ such as its antidiabetic, antioxidant, anticancer, hypolipidemic, and anti-inflammatory activities have been recognized. The present review focuses on the findings of recent studies on the protective effects of TQ against cardiovascular diseases. In the current review, we additionally concluded that TQ may be therapeutically effective agents for controlling diabetes and hyperlipidemia by decreasing the oxidative stress and inflammatory responses.

1. Introduction

1.1. General knowledge

Cardiovascular diseases (CVDs) are a group of disorders in the heart and blood vessels that are responsible for the death of approximately 23.6 million people around the world [1]. According to WHO reports, unhealthy diet, tobacco use, cigarette smoking, environmental pollution, physical inactivity, and alcohol addiction are the most important risk factors for developing CVDs [1]. However, improving quality of life by avoiding these factors has been shown to reduce the risk of CVDs. The consumption of healthy food with low in free sugar, salt, and fat, is rich with natural plant products may be one of the major effective factors for protection against CVDs [2]. Plants containing flavonoids have been used for the treatment of various illnesses in many years [3,4]. In the modern pharmacology, flavonoids are receiving much more attention as an effective treatment for CVDs due to its anti-inflammatory, antioxidant, and vasodilatory effects [5]. Thymoquinone (TQ) has several bioactive components [6]. It has been found in the seeds of *Nigella sativa*, a plant species belonging to the Ranzmculaceae family [6]. TQ was also observed in various plants belonging to the Lamiaceae including *Agastache*, *Coridothymus*, *Origanum*, *Monarda*, *Mosla*, *Satureja*, *Thymbra*, and *Thymus* [7–9]. It has also been found in genus *Telraclinis,* and in a glycosidic form in the genera of *Cupressus* and *Juniperus* of the Cupressaceae family [10]. Many plant species contain TQ with its dimeric and reduced forms dithymoquinone (DTQ) and thymohydroquinone (THQ) [11].

TQ, the major component of Lamiaceae family, exhibits protective effects against coronary artery diseases, respiratory failures, urinary system failures, hypertension, diabetes, nemodegenerative diseases, apoptosis, inflammation, and oxidative stress. The anti-oxidant and anti-inflammatory activities of TQ may cause its clinical effect against various diseases [12]. The anti-inflammatory effect of TQ is associated to its inhibitory effects on cyclooxygenase and 5-lipoxygenase and its antioxidant effect is associated with the scavenging activity against reactive oxygen species (ROS) [13]. TQ penetrates to physiological barriers and access to subcellular compartments, and exhibits the radical scavenging effects [14,15]. TQ also reacts with glutathione (GSH), NADH, and NADPH to form glutathionyl-dihydro-TQ (reduced species) [16] and combats

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with free radicals [17]. It has been observed that TQ possesses protective aspects for inhibition and treatment of CVDs [18]. The present review aims to increase our knowledge on the protective effects of TQ against CVDs by gathering the present scientific literature. In addition, the isolated TQ from Ranzmculaceae family may be used as an emerging potential therapeutic drug for treatment of CVDs in the future.

1.2. Pharmacokinetics properties

TQ (2-Isopropyl-5-methylbenzo-1, 4-quinone) is the most bioactive ingredients of seeds with molecular formula $C_{10}H_{12}O_2$ and molar mass 164.20 g/mol [19]. TQ comprises of the Enol, the keto and mixtures forms. The keto form is the major form that is involved in the pharmacological effects of TQ [19]. The hydrophobic property of TQ limits its bioavailability and drug formulation [19]. In the experimental studies showed effects of TQ in intravenous (*i.v.*) [20], intraperitoneal (*i.p.*) [21], and oral administrations [21]. After oral usage, TQ is metabolized via the liver metabolizing enzymes such as DT-diaphorase (a quinine reductase) that changes TQ into a reduced form THQ [22]. The clearance rate of TQ after intravenous administration was 7.19 mL/kg/min, and the estimated volume of distribution at steady state (Vss) was 700.90 mL/kg in animal models. Following oral adminstration the clearance rate was 12.30 mL/min/kg and Vss was 5 109.46 mL/kg. The elimination half-life (T1/2) of TQ was about 217 min [22]. In addition, the percentages of TQ-protein binding in human and rabbit plasma were 98.99 and 99.19 [22] which indicate the quick elimination and slow absorption of TQ following oral exposure. It has been shown that TQ causes complex formation with human serum albumin (HSA), bovine serum albumin (BSA), and α1-acid glycoprotein (AGP) in serum [23]. In addition, it was observed that the association between TQ and HSA as well as TQ and AGP does not affect the pharmacological properties of TQ. However, the covalent binding of TQ to BSA prevented the TQ anti-cancer activity against cancer cells [23]. The lack information on the bioavailability and pharmacokinetic of TQ limits its application in clinical trail. In recent years, the some analogs of TQ have been synthesized such as molecular micelle modified poly (d,l lactide-co-glycolide) (PLGA) nanoparticles, solid lipid nanoparticles (SLNs), TQ-encapsulated chitosan nanoparticles, TQ-loaded liposomes (TQ-LP), caryophyllyl, germacryl conjugates, fatty acid conjugates, and TQ-loaded nanostructured lipid carriers (TQ-NLCs). They may be affect on its bioavailability and application in clinical phase [23].

2. Methods

Various resources including Google Scholar, Scopus, PubMed, Web of Science, Science Direct, and Persian Websites (www.sid.ir) with keywords search of thymoquinone, cardiovascular diseases, diabetes, lipid profile and atherosclerosis have been selected in preparation of this review.

3. Cardioprotective effects of thymoquinone

CVDs are increasing rapidly worldwide. The alternation in lipid profiles (increased cholesterol and LDLC level); diabetes and hypertension are the major risk factors for CVDs [24]. The health protective effects of the TQ are discussed below (Table 1).

3.1. Protective effects of thymoquinone against atherosclerosis

Atherosclerosis is the main cause of CAD identified by a thickness in wall of arteries that is a result of the proliferation of intimal-smooth-muscle cell invasion and accumulation of white blood cells (foam cells) creating an atheromatous (fibrofatty) plaque [25]. Atherosclerotic plaque is made of cholesterol, oxidation of low density lipoprotein (Ox-LDL), fatty substances, cellular waste products, calcium, and fibrin that cause hardening and narrowing of the arteries [25]. Hypercholesterolemia is the main cause of the initiation and progress of atherosclerosis [26]. Inflammatory cells such as polymorphonuclear leukocytes and vascular endothelial cells are activated in hypercholesterolemia and causes overproduction of free radicals [18]. Free radicals are implicated in the initial and development stages of atherosclerosis by oxidation of Ox-LDL and contributed to the inflammatory state of atherosclerosis [18]. During the last decades, several studies have indicated that natural antioxidant compounds such as TQ ameliorated oxidative stress-induced atherosclerosis [27]. In these regards, Ragheb and co-worker (2008) indicated that TQ (20 mg/kg/d; through a nasogastric tube) decreased the oxidative stress, lipid profiles, and also prevented the progression of atherosclerosis in hypercholesterolemic animal. They reported that cholesterol-fed caused a decrease in the HDL-C level and also an increase of LDL-C, TC, and TG. However, TQ administration decreased protein carbonyls and the serum levels of LDL-C, TC/HDL-C, and TG, malondialdehyde (MDA) hypercholesterolemic rabbits. It is indicated that TQ improved the high cholesterol in the blood and prevented making plaque through decreasing oxidative stress and lipid profiles [28]. Nader and co-workers (2010) confirmed that TQ (3.5 mg/kg, *p.o.*) has protective effects on LDL-C, cholesterol and TG levels in the animals that received high lipid in their regime. They observed that TQ dramatically decreased LDL, total cholesterol, TG, and MDA levels, while increased HDL-C level through modulating oxidative stress [29]. Another study investigated the use of TQ for treatment of atherosclerosis in rabbits that were fed with a diet rich in cholesterol and also in combination with cyclosporine (CsA).They indicated that CsA aggravated intimal and media aortic lesions in rats, that were fed hypercholesterolemic diet by increasing cholesterol level and oxidative stress generation. However, TQ improved atherogenic plaque by modulating lipid levels and oxidative stress [30]. Recently, the reactive oxygen species (ROS) scavenger and hypocholesterolemic effects of TQ were studied in atherosclerotic rats. It was observed that TQ effectively improved cardiovascular disorders trough an enhancement in the activity of arylesterase accompanied by a decrease in activity of HMG-CoA reductase. TQ also inhibited the production of MDA, and shortened the lb-LDL, sd-LDL and LDL log times. This study showed that TQ ameliorated atherosclerotic process by inhibition of oxidative stress and modification of LDL [31].

3.2. Protective effects of thymoquinone against diabetes

Diabetes is a chronic metabolic disease that consists of progression in serum glucose and develops a deficiency of insulin caused by β-cell damage. Diabetes may be associated with serious complications, particularly cardiovascular diseases [32–34]. ROS plays an essential role in the progression of several diseases such as diabetes mellitus. Participation of ROS in the

Table 1
A summary of cardio protective effects of thymoquinone.

TQ dose	Rout of exposure	Experimental model	Effects	Ref
20 mg/kg	Gastric gavage	Hypercholesterolemic rabbit	Prevented the progression of atherosclerosis by decreasing LDL-C, TC/HDL-C, and TG, MDA levels and increasing HDL-C level	[28]
3.5 mg/kg	*p.o.*	Hypercholesterolemic rabbit	Prevented the progression of atherosclerosis by decreasing LDL-C, TC/HDL-C, and TG, MDA levels and increasing HDL-C level	[29]
10 mg/kg	*p.o.*	Hypercholesterolemic rabbit	Prevented the progression of atherosclerosis by decreasing LDL-C, TC/HDL-C, and TG, MDA and PC levels and also increasing HDL-C level	[30]
1 mL of 10 mg TQ	Gastric gavage	Hypercholesterolemic rat	Prevented the progression of atherosclerosis by enhancing in the activity of arylesterase, decreasing in the activity of HMG-CoA reductase, MDA level, and shortened the lb-LDL, sd-LDL and LDL log times.	[31]
5 mg/kg	*i.p.*	STZ-diabetic rat	Prevented the progression diabetes by decreasing the expression of COX-2 enzyme MDA levels and increasing the level of SOD in the pancreatic tissue.	[50]
20 mg/kg	*p.o.*	Diabetic mice during gestation and lactation	Prevented the progression diabetes in their offspring by decreasing in the levels of blood glucose, free radicals, plasma pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), and lipids, and restoring the number of circulating lymphocytes, the proliferation of SEB-stimulated lymphocytes and aberrant AKT phosphorylation	[51]
50 mg/kg	Gastric gavage	Gestational diabetic hamster	Prevented the progression diabetes by inhibiting the synthesis of gluconeogenic enzymes	[52]
80 mg/kg	Gastric gavage	STZ-diabetic rat	Prevented the progression diabetes by decreasing the activities of the glucose-6-phosphtse, fructose-1,6-bisphosphatase, MDA, and increasing the levels of GST, GPx, CAT, GSH, Vit E and Vit C	[53]
3 mg/mL	*i.p.*	STZ-diabetic rat	Prevented the progression diabetes and also improved the toxic activities of STZ, such as heterochromatin aggregates, DNA damage, segregated nucleoli, and fragmentation and vacuolization of mitochondria by decreasing the MDA level and increasing the SOD level	[54]
12.5, 25, 50 mg/kg	*p.o.*	Rat exposed to Iso	Prevented cardiotoxicity caused by Iso via decreasing TBARS level and increasing GSH/GSSG ratio of myocardial tissue, and plasma GR & SOD	[55]
50 mg/L	*p.o.*	Rat exposed to CP	Prevented cardiotoxicity caused by CP via decreasing serum TBARS, TNF-α, TC, TG, CK, LDH, Urea, and also increasing CAT, ATP, GPx, and GSH levels of the heart tissue	[56]
8 mg/kg	*p.o.*	Mice exposed to DOX	Prevented cardiotoxicity caused by DOX via decreasing serum CK and LDH	[57]

TQ: thymoquinone, STZ: streptozotocin, DOX: doxorubicin, Iso: isoproterenol, CP: cyclophosphamide, TG: triglycerides, TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, LDH: lactate dehydrogenase, CK: creatine kinase, COX-2: cyclooxygenase-2, HMG-CoA reductase: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, IL-6: interleukin-6, TNF-α: tumor necrosis factor-α, MDA: malondialdehyde, HDL-C: high density lipoprotein cholesterol, GSH: glutathione, SOD: superoxide dismutase, CAT: Catalase, GST: glutathione-S-transferase, GR: glutathione reductase, ATP: adenosine triphosphate, TBARS: thiobarbituric acid reactive substances, SEB: *Staphylococcus* enterotoxin B, *p.o.*: Per Os, *i.p.*: intraperitoneal.

pathophysiology of diabetes is manifested by the modification in lipid peroxides production, antioxidant enzymes, formation of oxidative stress, auto-oxidation of glucose, non-enzymatic protein glycosylation, and finally impaired glutathione metabolism [35–37]. In the last decade, there are several studies that evaluated the biological and medical properties of herbal remedies against diabetes [38,39]. It has been shown that *Nigella sativa* and its components involved in the alleviation of diabetes mellitus [40–42].TQ has anti-diabetic properties through decreasing ROS and therefore protecting the β-cell from injury [43–45]. Free radicals cause overgeneration of ROS that initiates several pathways related to the inflammatory signaling cascades which will lead to inflammation [46]. Activation of pro-inflammatory genes may induce inflammation in the local cells that might deteriorate the local cells, and finally causing the type 2 diabetes and β-cell apoptosis [47,48]. Therapeutic approaches were designed to eliminate oxidative stress and inflammation. Nowadays, new drugs were developed for the treatment of diabetes [49]. The protective effect of TQ against hyperglycemia has been shown in the STZ-diabetic rats. Al Wafai (2013) indicated that TQ reduces the activation of the COX-2 enzyme in the β-cells. Treatment with TQ also caused a decrease in the MDA levels and increase in the SOD (superoxide Dismutase) levels in the pancreatic tissue of diabetic rats [50]. It was also indicated that supplementation with TQ (20 mg/kg body weight/d, p.o.) during gestation and lactation of diabetic mice protected their offspring from diabetes and its complications via decreasing in the levels of blood glucose, free radicals, plasma pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), lipids, and also restoring the number of circulating lymphocytes,

proliferation of superantigen (SEB)-stimulated lymphocytes, and aberrant AKT phosphorylation [51].

TQ (50 mg/kg for 30 d, gastric gavage) decreased blood glucose levels in gestational diabetic hamster by inhibiting the synthesis of gluconeogenic enzymes [52]. Sankaranarayanan and Pari (2011) indicated that TQ (80 mg/kg, gastric gavage) ameliorated the glycemic effects of STZ plus nicotinamide in rats. TQ decreased the plasma glucose levels and also increased insulin levels by enhancing glucose utilization and decreasing hepatic glucose production. TQ reduced the activities of the gluconeogenic enzymes glucose-6-phosphatase and fructose-1, 6-bisphosphatase [53]. They also investigated the activity of TQ on lipid peroxidation, tissue antioxidant, and insulin secretion in the diabetic rats. TQ ameliorated the decreased the level of glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT), GSH, Vitamin E, Vitamin C, and also the increased levels of lipid peroxidation. These finding showed that TQ has the amelioration effects on β cell action and increase free radicals via its antioxidant characteristics [53]. Abdelmeguid *et al.*, 2010 indicated that TQ decrease the glucose level and also increased pancreatic insulin release in diabetic rats. TQ improved the toxic activities of STZ, such as heterochromatin aggregates, DNA damage, segregated nucleoli, fragmentation and vacuolization of mitochondria by modulating oxidative stress. These findings suggested that TQ ameilorates diabetes by protecting beta-cells from oxidative damage. Therefore, the antioxidant effect of TQ may improve the damaged beta-cells caused by hyperglycemia through down-regulation of inflammatory activity and oxidative stress [54]. These scientific findings indicated that TQ may be effective as an anti-diabetic agent in traditional medicine; however, the knowledge on protective effect of TQ is not sufficient especially on diabetes.

3.3. Protective effect of thymoquinone against chemical cardiotoxic agents

3.3.1. Isoproterenol

The therapeutic aspect of TQ (12.5, 25, 50 mg/kg, *p.o.*) has been investigated against isoproterenol caused myocardial damage. TQ treatment improved the histological modifications, the GSH/GSSG ratio of myocardial, plasma LDH, TBARS, GR and SOD induced by isoproterenol. This study indicated that TQ inhibits cardiotoxicity caused by isoproterenol via enhancing antioxidant content [55].

3.3.2. Cyclophosphamide

The cardioprotective effect of TQ (50 mg/L in water, *p.o.*) against cyclophosphamide has been studied. Cyclophosphamide increased serum cholesterol, TNF-α, TG, CK, LDH, Urea, and creatinine. In addition, cyclophosphamide increased total nitrate/nitrite and TBARS and also decreased CAT, ATP, GPx, and GSH levels of the heart tissue. However, TQ ameliorated the biological alterations caused by cyclophosphamide. Therefore TQ improved CP-induced cardiotoxicity by modulating nitrosative, oxidative stress and ameliorating the mitochondrial function [56].

3.3.3. Doxorubicin

Doxorubicin (DOX), an antitumor agent, may be disturbing cardiovascular function. One study indicated that TQ (8 mg/kg/d, *p.o.*) ameliorates cardiotoxic effects of DOX. They indicated that TQ decreases serum LDH and CK levels. They showed that TQ ameliorates cardiotoxicity without changing DOX antitumor effect [57].

4. Conclusions

The current review article shows the therapeutic properties of TQ on oxidative and inflammatory responses in cardiovascular diseases. TQ exhibits antioxidant and anti-inflammatory effects on various abnormalities associated with oxidative stress imbalance and inflammation. In addition, TQ acts as an agent with antidotal effects in several diseases caused by toxic agents. TQ improved diabetes and hyperlipidemia through increasing antioxidant and decreasing lipid peroxidation in serum and pancreatic tissue. The most protective properties of TQ against cardiovascular diseases may be related to its antioxidant and anti-inflammatory activities. Cardioprotective effects of TQ have been showed *in vivo* and *in vitro*, however, these protective effects have not been confirmed by clinical trials yet and more safety assessments are necessary to determine toxic properties of TQ in humans for long-term application. In addition, more studies should be conducted to confirm its traditional use as a treatment against cardiovascular diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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