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Review

Thymoquinone and its therapeutic potentials

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ABSTRACT

Herbal medicine has attracted great attention in the recent years and is increasingly used as alternatives to chemical drugs. Several lines of evidence support the positive impact of medicinal plants in the prevention and cure of a wide range of diseases. Thymoquinone (TQ) is the most abundant constituent of the volatile oil of *Nigella sativa* seeds and most properties of *N. sativa* are mainly attributed to TQ. A number of pharmacological actions of TQ have been investigated including anti-oxidant, anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial and anti-tumor effects. It has also gastroprotective, hepatoprotective, nephroprotective and neuroprotective activities. In addition, positive effects of TQ in cardiovascular disorders, diabetes, reproductive disorders and respiratory ailments, as well as in the treatment of bone complications as well as fibrosis have been shown. In addition, a large body of data shows that TQ has very low adverse effects and no serious toxicity.

More recently, a great deal of attention has been given to this dietary phytochemical with an increasing interest to investigate it in pre-clinical and clinical researches for assessing its health benefits. Here we report on and analyze numerous properties of the active ingredient of *N. sativa* seeds, TQ, in the context

Abbreviations: AGP, α1-acid glycoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, adenosine triphosphate; BMP, bone morphogenetic protein; BSA, bovine serum albumin; CAT, catalase; CDK, cyclin-dependent kinase; COX, cyclooxygenase; EAE, experimental allergic encephalitis; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GSH, glutathione; GSH-Px, glutathione peroxidase; GST, glutathione-S-transferase; HDL, high-density lipoprotein; JAK, janus kinase; JNK, Jun-N-terminal kinase; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Mcl-1, myeloid cell leukemia sequence 1; MDA, malondialdehyde; MMP, matrix metalloproteinase; MPO, myeloperoxidase; NADH, nicotinamide adenine dineucleotide; NADPH, nicotinamide adenine dineucleotide phosphate; NDEA, N-nitrosodiethylamine; NF-κB, nuclear factor-kappaB; NLRP3, nucleotide binding domain and leucine rich repeat containing family pyrin domain containing 3; NO, nitric oxide; Nrf2, nuclear factor erythroid derived 2-related factor 2; OVA, ovalbumin; PAF, platelet-activating factor; PARP, poly ADP-ribose polymerase; PGD2, prostaglandin D2; PGE2, prostaglandin E2; PI3K, phosphatidylinositol 3-kinase; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TBARS, thiobarbituric acid reactive substances; TGF-β, transforming growth factor-beta; Th-1 and Th-2, helper T-cells; TIMP-1, tissue inhibitor of metalloproteinase-1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; TQ, thymoquinone; VEGF, vascular endothelial growth factor; α-SMA, alpha-smooth muscle actin.

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of its therapeutic potentials for a wide range of illnesses. We also summarize the drug's possible mechanisms of action. The evidence reported sugests that TQ should be developed as a novel drug in clinical trials.

Contents

1. Introduction

Plants are invaluable sources of new drugs and interest in medicinal plants has increased due to increased efficiency of new plant-derived drugs. In addition, because of the concerns about the side effects of conventional medicine, the use of natural products as a replacement for the use of synthetic drugs has risen considerably in the last decades. The wide utilization of herbal drugs has encouraged scientists to investigate their impressive effects on health, and a large number of medicinal plants and their active extracted ingredients are extensively studied for their potentials to protect cells from injuries.

Among the promising medicinal plants is *Nigella sativa* (also called black cumin, black seed). *N. sativa* Linn as a member of the botanical family of Ranunculaceae is an annual herbaceous [1]. It has illustrious and religious background and its seeds and oil have been commonly used as a traditional remedy to treat a variety of health conditions for more than 2000 years [2]. Black seed is known as the curative black cumin in the Holy Bible and is described as the Melanthion of Hippocrates and Discroides and as the Gith of Pliny [3]. Furthermore, the Prophet Muhammad (PBUH) advised: "Hold onto use the black cumin, because it can heal every disease except death" [2].

The seeds, which are the rich source of the active ingredients of plant, have long been used in the Middle and Far East as a traditional medicine for a wide range of pathological conditions [4]. It is used in ethnomedicine to treat ailments and symptoms including, asthma, bronchitis, inflammation, eczema, fever, influenza, hypertension, cough, headache, dizziness, diabetes, kidney and liver dysfunctions, nervous disorders, rheumatism, cancer and related inflammatory diseases, gastrointestinal problems, and overall for general well-being [1,4,5].

N. sativa seeds (NS) contain diverse but well-characterized chemical components; they include both fixed and essential (volatile) oil, proteins and amino acids, carbohydrates, alkaloids, organic acids, saponins, crude fibers, vitamins, and minerals [6].

Thymoquinone (2-isopropyl-5-methylbenzo-1, 4-quinone) (TQ), the most abundant constituent of the volatile oil also present in the fixed oil, is the biologically active compound of NS seeds [1]. TQ as a bioactive component is found in many medicinal plants. Besides Ranunculaceae, the presence of this compound has been confirmed in several genera of the Lamiaceae family such as *Monarda*, and the Cupressaceae family such as *Juniperus* [7]. Although, TQ holds a great potential as an anti-oxidant and an anti-inflammatory agent, it also has a vast array of other benefits. TQ, as a naturally derived agent, has lately received particular consideration and has been extensively studied for its therapeutic properties.

In this review, we focus on the therapeutic potential of TQ in disease conditions. The evidence we report suggests that TQ should be investigated further in clinical trials.

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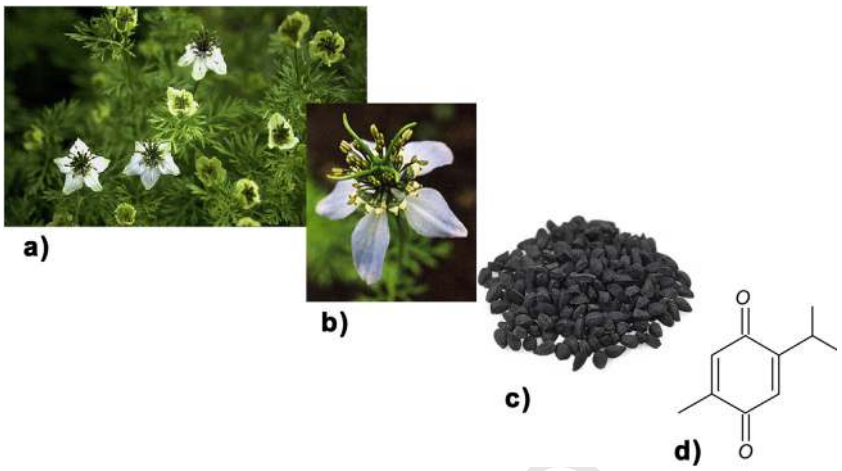

Fig. 1. The *Nigella sativa* plant (a), its flower (b) and seeds (c); and the chemical structure of bioactive component of seeds, thymoquinone (TQ) (d).

Table 1
The significant properties of thymoquinone (TQ).

Systematic/IUPAC name	2-Isopropyl-5-methylbenzo-1,4-quinone
Molecular formula	$C_{10}H_{12}O_2$
Molar mass	164.20 g mol^{-1}
Appearance	Crystalline and dark yellow
CAS number	490-91-5
PubChem CID	10281

1.1. Pharmacological properties

The chemical composition of NS seeds is rich, and diverse active chemical components have been isolated from them. The seeds were shown to contain a fixed oil (>30%, wt/wt) and a volatile oil (0.40–0.45%) [8]. The active principles include thymoquinone, thymohydroquinone, dithymoquinone (nigellone), thymol, carvacrol, nigellicine, nigellidine and α-hedrin [9]. Nevertheless, most of the known actions have been attributed to TQ.

TQ is the most bioactive component of seeds also of the volatile oil (18.4–24%) [1], it was first extracted by El-Dakhakhny [10] using thin layer chromatography on silica gel. Illustration of NS plant, seeds of plant and the chemical structure of TQ are presented in Fig. 1 and the most relevant properties of TQ are summarized in Table 1.

TQ exists in tautomeric forms including the enol form, the keto form and mixtures. The keto form being the major fraction (~90%), and is responsible for the pharmacological properties of this compound [4]. TQ is a hydrophobic molecule, thus its solubility is a challenge that could affect its bioavailability and cause limitations in drug formulation. In addition, it's solubility dependent on the time as its solubility ranges from 549 to 669 μg/ml in aqueous solutions at 24 h, increasing at 72 h to 665–740 μg/ml [11]. Therefore, recently attempts have been made to synthesize novel analogs of TQ with enhanced bioavailability and activity. Some synthetic analogs of TQ and their effects on disease models are presented in Table 2.

There are different routes for administration of TQ including intravenous (*i.v.*) [18,19], intraperitoneal (*i.p.*) [20–22], and oral subacute and subchronic administration [20,23–26]. However, oral administration could lead to biotransformation due to the metabolizing activity of the liver enzymes such as DT-diaphorase (a quinine reductase) that catalyzes reduction of TQ into a hydroquinone [27].

El-Dakhakhany [28] reported an LD_{50} of 10 mg/kg for TQ when injected intraperitoneally in rats. Another study reported that doses of 4, 8, 12.5, 25 and 50 mg/kg *i.p.* in mice did not alter the biochemical parameters, including serum alanine transaminase (ALT) and lactate dehydrogenase (LDH) [29]. In the same study, doses of TQ higher than 50 mg/kg body weight by intraperitoneal injection were lethal to mice and the LD_{50} was 90.3 mg/kg [29]. In many other studies conducted to determine anti-inflammatory, anti-cancer, anti-oxidant and cytoprotective effects of TQ the investigators have used doses between 5 and 12.5 mg/kg, injected intraperitoneally in mice and rats without important toxicity [24,29–31]. A number of studies administered oral TQ dose in the range of 10–100 mg/kg body weight without any reported toxic or lethal effects [32–36]. The maximum tolerated dose for intraperitoneal injection was 22.5 mg/kg in male and 15 mg/kg in female rats, whereas for oral ingestion it was 250 mg/kg, in both male and female rats [37].

The calculated clearance of TQ following intravenous administration was 7.19 ml/kg/min, and the estimated volume of distribution at steady state (*Vss*) was 700.90 ml/kg. At variance, after oral administration the apparent clearance value was 12.30 ml/min/kg and *Vss* was 5109.46 ml/kg. The calculated absorption half-life ($T_{1/2}$) of TQ was about 217 min, and it was rapidly removed from plasma [38]. The UV–vis spectra of TQ had one sharp peak (λ_{max}) at 254–257 nm [11]. TQ was highly sensitive to light, even after a short period of exposure. A short period of light exposure led to severe degradation, independent of the solution pH and

Table 2
Some analogs have been developed for enhanced bioavailability and activity of TQ.

Types	Disease models	Refs
Molecular micelle modified poly (D,L lactide-co-glycolide) (PLGA) nanoparticles	MDA-MB-231 breast cancer cells	[12]
Solid lipid nanoparticles (SLNs)	Paracetamol-induced liver fibrosis and/or cirrhosis	[13]
TQ-encapsulated chitosan nanoparticles	Brain diseases	[14]
TQ-loaded liposomes (TQ-LP)	MCF-7 and T47D breast cancer cell lines	[15]
Caryophyllyl and germacryl conjugates as well as fatty acid conjugates	HL-60 human leukemia, multidrug-resistant KB-V1/Vbl cervix, 518A2 melanoma and MCF-7/Topo breast cancer cells	[16]
TQ-loaded nanostructured lipid carriers (TQ-NLCs)	Gastroprotective effects and inhibition of the formation of ethanol-induced ulcers	[17]

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solvent type. In addition, it was unstable in aqueous solutions, particularly at an alkaline pH. TQ stability decreased with rising pH; at alkaline pH, it suffered the highest degradation rate with the minimal degradation being at acidic pH [11].

Protein–drug interactions are an important factor on the pharmacokinetics and pharmacological properties of drugs. The estimated percentages of TQ-protein binding in rabbit and human plasma were 99.19 and 98.99, respectively [38]. Therefore, TQ represented a compound with quick elimination and relatively slower absorption after oral administration.

Lupidi et al. evaluated the interactions between TQ and human serum albumin (HSA). They showed that the association between TQ and HSA does not affect the secondary structure of HSA. The thermodynamic analysis of the HSA/TQ complex formation showed that the binding process was spontaneous and the hydrophobic interactions were main intermolecular forces stabilizing the complex [39]. In addition, El-Najjar et al. [40] studied the effect of TQ binding with bovine serum albumin (BSA) and α1-acid glycoprotein (AGP) on its anti-cancer activity. The results suggested that covalent binding of TQ to BSA led to losing the TQ anti-cancer activity against tested cancer cells, but the TQ anti-cancer effects was not affected when it is bound to AGP.

The lack of bioavailability and pharmacokinetic parameters, and formulation problems delayed the usage of TQ in clinical phase. Hence, more investigation is needed for a better understanding of TQ pharmacological properties meant for future clinical development.

1.2. Anti-oxidative, anti-inflammatory and immunomodulatory activities

Currently, the use of natural anti-oxidant compounds and phytochemicals as a therapy in diseases related to oxidative stress has gained massive interest for their abilities to quench free radicals and the protection of body against oxidative stress-induced pathogenesis. Furthermore, natural immunomodulators help the body by stimulate and strengthen the immune system. In this regard, TQ as a natural compound has become an imperative agent in pharmacological toxicity studies due its potent anti-oxidant, anti-inflammatory, and immune enhancement capacity [4].

Anti-oxidant enzymes are essential part of the cellular defense against reactive oxygen species (ROS). Reactive oxygen species such as superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical (•OH) and peroxyl radical (ROO•) are continually generated in cells by aerobic metabolism also by exogenous sources such as UV radiation and environmental pollution. Generation of ROS induces oxidization of biomolecules including membrane lipids, proteins and nucleic acids.

TQ is a potent phytochemical anti-oxidant due to the scavenging activity against several ROS including superoxide anion, hydroxyl radical and singlet molecular oxygen [32,41], and thus it can antagonize the adverse effects resulting from elevated ROS levels in various disorders.

It has been shown that TQ and tert-butylhydroquinone (TBHQ), a synthetic structurally-related, powerfully inhibited iron-dependent microsomal lipid peroxidation [42]. The anti-oxidative potential of TQ may be related to the redox properties of the quinone structure of molecule and unrestricted ability of TQ to cross from physiological barriers and easy access to subcellular compartments, all of which help the radical scavenging effects [42,43].

Under physiological conditions, TQ reacts with glutathione (GSH), NADH and NADPH through a spontaneous reaction to form reduced species, glutathionyl-dihydro-TQ, after rapid reaction with GSH, and dihydrothymoquinone, after slow reaction with NADH and NADPH [44]. TQ may undergo a two-step process of one-electron reduction by microsomal NADPH CYP reductase, NADH CYP-b5 reductase or NADH-ubiquinone oxidoreductase, or a one-step two-electron reduction by NADPH-quinone oxidoreductase to produce thymohydroquinone [44] (Fig. 2). When these metabolites are formed they can remove free radicals, an effect that can replace the endogenous anti-oxidant defense molecules, *viz.* GSH and superoxide dismutase (SOD), and prevent lipid peroxidation. These forms were highly reactive toward different redox states of hemoglobin and myoglobin that led to recovery of these from oxidative stress [45].

Anti-oxidant enzymes, such as GSH, SOD, catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GSH-Px) constitute the main pool of the anti-oxidant system of most cells. It is well known that anti-oxidant enzymes are responsible for neutralizing the free radical-induced oxidative damage [46]. TQ induced the expression and/or activity of GST [47–49], GSH-Px [49], SOD [32,42,48], and glutathione reductase [49]. TQ improved the plasma and liver anti-oxidant capacity and expression of liver anti-oxidant genes. In models with induced hypercholesterolemia it can up-regulate the genes coding for GST, GSH-Px and CAT leading to elevation of hepatic levels of these enzyme to overcome oxidative stress induced during diethylnitrosamine metabolism [27,50].

The generation of free oxygen radicals is one of the possible mechanisms by which several drugs exert their toxicity. TQ had protective effects on several organs against oxidative damage induced by a variety of free radical-generating agents and it had also cytoprotective properties. It protected kidney from cisplatin [30], doxorubicin [51], gentamicin [52], vancomycin [53] and mercuric chloride [54]-induced nephrotoxicity; and liver from carbon tetrachloride [24,29], cyclophosphamide [55], acetaminophen [56] and aflatoxin B1 (AFB1) [57]-induced hepatotoxicity. Moreover, it showed that TQ protected heart from doxorubicin [26,41] and cyclophosphamide [58]-induced toxicity. It also alleviated lung injuries induced by cyclophosphamide [59], toluene [36] and bleomycin [60]. TQ has been shown to enhance detoxification and inhibit benzo(a)pyrene-induced fore-stomach tumors [61]. It had also protective role in the prevention of gentamicin ototoxicity [62].

TQ pre-treatment (5 mg/kg) also restored completely 1,2-dimethyl-hydrazine (DMH)-induced oxidative stress, which occurs in initiation stage of colon tumor. TQ corrected the oxidative status and malondialdehyde (MDA), CAT, SOD and GST-Px level at promotion, with a reduction in tumor incidence and dysplasia degree [63]. TQ has been shown to have anti-oxidative and anti-inflammatory efficacy in hippocampal neurodegeneration model after chronic toluene exposure [33]. TQ ameliorated most of the toxic effects related to streptozotocin (STZ) and preserved beta-cell integrity by decreasing oxidative stress [64]. NS oil and TQ have anti-oxidant properties also in radiation-injured brain tissue, especially against nitrosative stress in the brain tissue of the irradiated rats [65].

TQ exhibited anti-oxidant/anti-inflammatory activities in experimental models, thus may be a clinically viable agent against a variety of inflammatory conditions. Inflammation as a part of the complex biological response to harmful stimuli is mainly mediated by two enzymes, cyclooxygenase and lipoxygenase, which generate prostaglandins and leukotrienes, respectively [41]. Houghton et al. [31] showed that the anti-inflammatory action of TQ resulted from prevention of eicosanoids generation, such as thromboxane B2 and leukotriene (LT) B4, by inhibiting both cyclooxygenase and 5-lipoxygenase, and in part *via* non-enzymatic peroxidation of membrane lipids. TQ induced a significant inhibition on LTC4 synthase activity [66]. Furthermore, *in vitro* treatment of calcium- or ionophore-stimulated neutrophils with TQ inhibited 5-lipoxygenase products and 5-hydroxy-eicosa-tetra-enoic acid production [67].

It was a potent anti-inflammatory agent in an asthmatic murine model [68]. It was a strong inhibitor of inflammatory cell aggregation in bronchoalveolar lavage (BAL) fluid and lung tissues, and of

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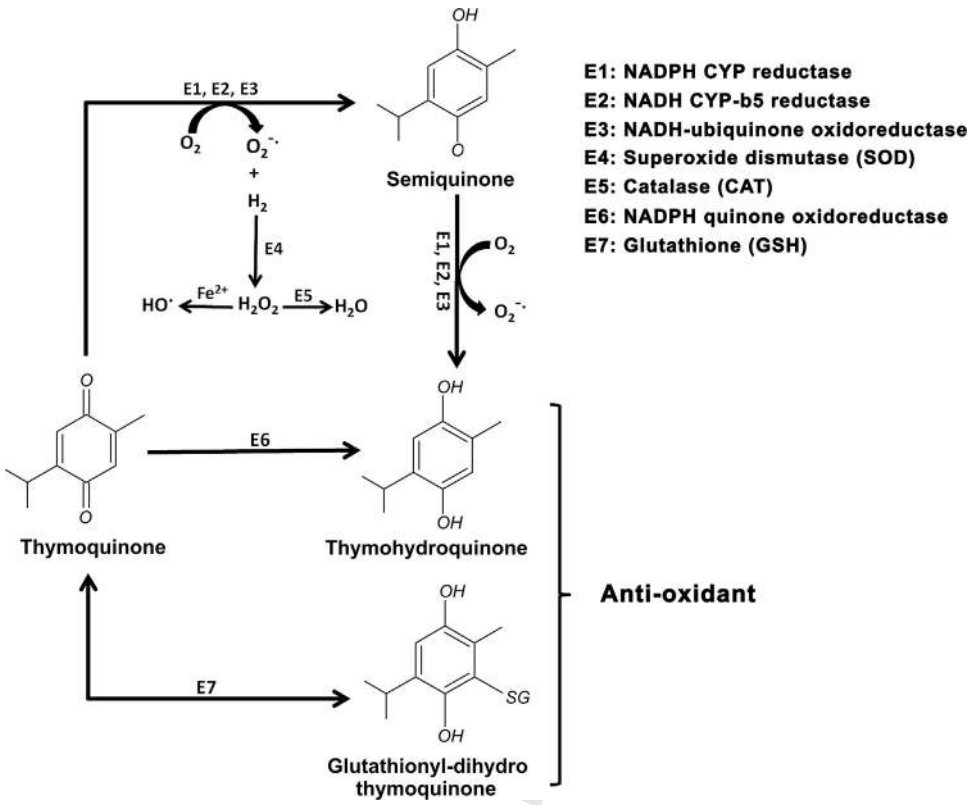

Fig. 2. Mechanism of oxido-reduction cycling of TQ. In enzymatic reaction by a two-step one-electron reduction, or by a one-step two-electron reduction converts TQ into thymohydroquinone. In one electron reduction of TQ, microsomal NADPH cytochrome P450 reductase, microsomal NADH cytochrome-b5 reductase, and mitochondrial NADH ubiquinone oxidoreductase, catalyze the conversion of TQ to semiquinone. Subsequently, semiquinone is converted into thymohydroquinone. Alternatively, a one-step reaction causing direct formation of thymohydroquinone may take place. TQ may also be reduced in a non-enzymatic reaction through interaction with GSH to generate glutathionyl-dihydro-TQ. The reduced products of TQ, glutathionyl-dihydro-TQ and thymohydroquinone, is known for its anti-oxidant activity. A semiquinone produced by one-electron reduction can contribute to the pro-oxidant effect of TQ in the tumor environment. Superoxide anion generated through oxidation of reduced TQ can be detoxified by SOD and CAT. In the absence of detoxificating enzymes, which is common in numerous cancers, the accumulation of superoxide may contribute to the pro-oxidant effect of TQ.

the expression of TGF-β1 and inducible nitric oxide (NO) synthase (iNOS) mRNA, all converging in explaining its potency in antagonizing airway inflammation [68]. El Gazzar et al. [21] showed the anti-inflammatory effect of TQ in a mouse model of allergic lung inflammation. TQ attenuated pulmonary inflammation in allergic asthma by decreasing lung eosinophilia, and Th2 cytokines and inflammatory cell infiltration in the lung. It also decreased the elevated levels of ovalbumin (OVA)-specific IgE and IgG1 in serum. It inhibited significantly allergen-induced lung eosinophilic inflammation and represented an inhibitory influence on IL-4, IL-5 and IL-13, with some effect in inducing IFN-γ production in the BAL fluid [21]. Administration of TQ also down-regulated 5-lipoxygenase expression, and LTB4 and LTC4 production by lung cells in airway inflammation [69]. In addition, TQ inhibited COX-2 expression in a mouse model of OVA-induced allergic airway inflammation [70]. Administration of TQ significantly reduced the ocular symptoms in allergic conjunctivitis by attenuating the recruitment of eosinophils and reducing the levels of IgE, histamine and cytokines. Furthermore, it blunted in mice immunized and exposed to OVA the mRNA expression and serum level of interleukins including IL-4, -5 and -13, as well TGF-β [71].

In arthritis-induced animal models, the administration of TQ was effective against rheumatoid arthritis [72] and autoimmune encephalomyelitis [73]. Umar et al. [72] have demonstrated the anti-arthritic effect of TQ in collagen induced arthritis. They found that TQ suppressed the increase of NO and myeloperoxidase (MPO), along with enhanced the activity of GSH, CAT and SOD. Moreover, TQ abolished the accumulation and activation of polymorphonuclear cells, and maintained the homeostasis in the cytokine imbalance. Tekeoglu et al. [74] reported that administration of TQ had an action similar to methotrexate in adjuvant-induced arthritis in rats. Furthermore, Vaillancourt et al. reported that TQ (5 mg/kg/day) significantly reduced the serum levels of 4-hydroxynonenal, IL-1β and TNF-α, as well as of a number of factors known to be involved in rheumatoid arthritis pathogenesis, such as alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase. The protective effects of TQ against rheumatoid arthritis were also evident from the decrease in arthritis scoring and bone histology [75]. Increase in the levels of pro-inflammatory mediators including IL-1β, IL-6, TNF-α, IFN-γ and PGE2, and decreased level of IL-10 was reported in arthritic rats, which were corrected with TQ [69].

Experimental allergic encephalitis (EAE) as a T-cell mediated autoimmune disease is a widely accepted animal model for the human multiple sclerosis (MS). The EAE rats treated with TQ showed higher GSH level, no perivascular inflammation and no disease symptoms compared with EAE untreated animals. Furthermore, TQ treatment inhibited NF-κB activation in encephalomyelitis in rats [76]. By these data, TQ was shown to have an anti-oxidative stress effect leading to therapeutic effects in EAE animals [73].

TQ attenuated the pro-inflammatory and oxidative responses mainly by modulating NF-κB and TNF-α production [77]. In addition to significant down-regulation of TNF-α, IL-1β and COX-2, TQ also inhibited the constitutive TNF-α-mediated activation of NF-κB and reduced the transport of NF-κB from the cytosol to the nucleus

[78]. Furthermore, it inhibited lipopolysaccharide (LPS)-induced TNF-α production in the rat basophil cell line, RBL-2H3 by a mechanism involving NF-κB. Experimental studies have reported that TQ reduced TNF-α, interleuken-6 and IL-1β in blood and tissues, and protected tissues by reducing inflammation [74,75]. TQ inhibited IL-6 signaling [79]; and it is possible that the role of TQ in EAE [76], rheumatoid arthritis [72], allergic asthma [69], allergic lung inflammation [21], experimental colitis [80], and immunomodulatory effects [4] is due to suppression of IL-6 signaling. Furthermore, TQ suppressed NO overproduction and iNOS expression induced by different agents in various animal tissues [20,48,81–83]. It diminished iNOS levels in LPS-activated rat peritoneal macrophages and thus suppressed the production of NO by macrophages [22,84], an effect that may be useful in ameliorating the inflammatory and autoimmune conditions. TQ also acted *in vitro* as inhibitor of COX-1 and -2 [85], also inhibiting p38 in murine macrophages [22].

The immunomodulatory effect of TQ was observed also in mixed lymphocyte cultures, where it caused decreased secretion in the levels of the cytokines IL-1β and -8 [86]. It enhanced the survival and activity of antigen-specific CD8T cells thus protecting against tumor growth [87]. TQ might regulate immunity also by influencing dendritic cell functions such as maturation, cell pH (by affecting Na^+/H^+ activity), oxidative burst, migration and cytokine release and survival. Dendritic cell volume may also be affected by TQ [88]. TQ may enhance the activation of Nrf2, thereby raising the expression of heme oxygenase-1 (HO-1). TQ induced HO-1 expression in HaCaT cells by activating Nrf2 through ROS mediated phosphorylation of Akt and adenosine monophosphate-activated protein kinase (AMPK) as upstream targets [89].

In addition to TQ, components from other herbs are effective on HO-1 and Nrf2. A chemically synthesized kavalactone derivative, which isolated from *Piper methysticum*, is capable of inhibiting LPS-stimulated iNOS induction and NO production *via* activation of Nrf2 signaling and HO-1 induction in microglial cells [90]. Some small activators of Nrf2/HO-1 such as carnosol, a phenolic diterpene in *Rosmarinus officinalis* and supercurcumin are effective modulators of inflammation, and up-regulate HO-1 and down-regulate the inflammatory response TNF-α, PGE2 and nitrite [91]. Consequently, activation of Nrf2 and HO-1 moderate oxidative stress and inflammation. Thus, anti-inflammatory and anti-carcinogenesis properties of TQ, in part, can be the result of induction of Nrf2 and HO-1 expression.

Reinforcement of body's anti-oxidant store is believed to inhibit oxidative stress-induced tissue damage and neoplastic transformation of cells, and have therapeutic relevance; hence TQ in therapeutic perspective as a cytoprotective agent against oxidative damage, autoimmune and other inflammatory complications in many tissues may be promising option.

1.3. Anti-cancer effects

Among the novel anti-neoplastic drugs that are currently under investigation, bioactive natural products have gained considerable attention. Natural compounds and phytochemicals may be exceptional resource for anti-cancer agents, and may be valuable alternatives to synthetic drugs in cancer therapy, or used to enhance their effect and reduce their dosing and limiting their toxicity [92]. An ideal cancer therapeutic agent is one that exerts its anti-cancer effect with partial cytotoxicity over normal tissues. One of the advantages of TQ is that its anti-cancer effects has been shown to be activated more specifically against cancer cells than normal cells [93]. As detailed below, among a wide spectrum of phytochemicals TQ is a compound exhibiting promising anti-carcinogenic, anti-neoplastic, anti-proliferative and anti-mutagenic activities against miscellaneous tumor models. It also is chemopreventive, and reduces the toxic effects of standard anti-neoplastic agents, as well was a chemosensitizer when used in combination with chemotherapeutic agents, while it was minimally toxic to normal cells.

TQ was shown to have a dual role, and depending on the cellular microenvironment, it may act as an anti-oxidant or as a pro-oxidant. It exerted its biological functions by ROS generation in tumor cells where it acted as pro-oxidant. TQ reacted with amino or thiol groups of amino acids, undergone a series of oxido-reduction reactions, and then metabolized to semiquinone or thymohydroquinone, ultimately leading to the production of ROS [94].

TQ inhibited the growth of the breast cancer cell lines MCF-7, MDA-MB-231 and BT-474, exerted strong anti-proliferative activity, and increased the cytotoxicity of doxorubicin (DOX) and 5-fluorouracil (5-FU), when combined with them. It showed that the anti-cancer effects of TQ resulted from the ability of the compound to increase PPAR-γ activity and to down-regulate the expression of pro-apoptotic genes including Bcl-2, Bcl-xL and survivin [95]. TQ also induced cell cycle arrest by suppression of cyclin D1, cyclin E, and the cyclin dependent kinase (CDK) inhibitor p27 expression in T-47D and MDA-MB-468 breast cancer cells. TQ induced cell cycle arrest in G1 phase during initial time incubation, while extended exposure to TQ resulted in loss of mitochondrial membrane potential thus inducing apoptosis through release of cytochrome c and interfering Akt activation [96].

It also inhibited DOX-resistant MCF-7/DOX cell proliferation. TQ arrested MCF-7/DOX cells at G2/M phase and increased cellular levels of p53 and p21 proteins. TQ-induced apoptosis was associated with changes in mitochondrial membrane potential, activation of caspases and PARP cleavage in MCF-7/DOX cells. In addition, TQ treatment increased Bax/Bcl2 ratio *via* up-regulating Bax and down-regulating Bcl2 proteins [97]. In addition, it was douns to be a radiosensitizer, such that using TQ in combination with ionizing radiation such as γ-radiation (2.5 Gy) was found to exert a synergistic cytotoxicity against breast cancer cells [98].

TQ was cytotoxic toward SiHa cells, human cervical squamous carcinoma, with even higher efficacy when compared to cisplatin in a pathway involving elevation of the p53 activity and the Bax/Bcl-2 ratio [99]. TQ has also shown cytotoxicity against BG-1 and human ovarian adenocarcinoma cells [93]. The cytotoxicity of TQ has been reported in PC3 prostate cancer cell xenograft tumor growing in SCID mice. TQ (6 mg/kg daily for 15 days) inhibited tumor angiogenesis and tumor growth by suppressing Akt and extracellular signal-regulated signaling pathways. It also significantly potentiated the apoptotic effects of thalidomide and bortezomib [100]. TQ (at the dose of 20 mg/kg) repressed hormone-refractory prostate cancer, by suppressing the expression of androgen receptor and the transcription factor E2F-1 [101].

Intragastric administration of TQ induced growth inhibition and apoptosis in the human osteosarcoma cell line SaOS-2, and blocked human umbilical vein endothelial cell (HUVEC) tube formation. TQ significantly down-regulated NF-κB DNA-binding activity, as well as the expression of XIAP, survivin and vascular endothelial growth factor (VEGF) in SaOS-2 cells, while up-regulating the expression of cleaved caspase-3 and Smac. Overall, TQ effectively inhibited tumor growth and angiogenesis by inhibition of NF-κB and downstream effector molecules in osteosarcoma [102].

The effect of TQ on M059K and M059J human glioblastoma cells was reported. Cytotoxicity of TQ resulted from induction of telomere shortening, DNA damage and apoptosis in these cells [103]. It was also demonstrated that TQ modulates potently proteasome complex activity in U87 MG and T98G malignant glioma cells. Inhibition of this complex led to intracellular accumulation of p53 and Bax in malignant cells, and may be linked to accumulation of ubiquitin conjugates at the onset of apoptotic events [104]. In addition, TQ may have anti-metastatic activities since it inhibited *in vitro* migration, adhesion and invasion of human glioblastoma

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U87 and CCF-STTG1 cells. At the molecular level, this was mediated by a drastic down-regulation of Focal Adhesion Kinase (FAK), associated with a reduction of extracellular signal-regulated kinase (ERK) phosphorylation as well matrix metalloproteinase (MMP)-2 and -9 secretion [105]. TQ also was found to be cytotoxic in the mouse neuroblastoma cell line Neuro-2a, where it triggered apoptosis as evidenced by elevation of Bax/Bcl2 ratio, cytochrome c release from mitochondria, activation of caspases-3 and -9, and down-regulation of XIAP [106].

Studies demonstrated the anti-cancer effect of TQ in colorectal carcinoma [107], a multistep process, in which oxygen radicals play a critical role during the initiation, promotion, and progression phases. This agent can abrogate the stress response pathway sensor CHEK1 and contribute to apoptosis in colorectal cancer cells [108]. Furthermore, in a HCT116 colorectal cancer cells xenograft model, a three-time weekly intraperitoneal injection of 20 mg/kg TQ reduced the tumor size. This occurred through TQ-induced apoptosis involving an up-regulation of both p53 and p21 expression [108]. It inhibited also the proliferation of human colon cancer cells Caco-2, HCT-116, LoVo, DLD-1 and HT-29 by increasing the phosphorylation states of the mitogen-activated protein kinases (MAPK), JNK and ERK [109]. TQ was efficacious in protecting and curing DMH-induced initiation phase of colon cancer, while exerting a protective role at promotion. These effects may be related to its capacity in prevention of DMH-induced oxidative stress [63]. TQ (orally and daily 375 mg/kg body weight for 12 weeks) decreased the number of large polyps in the small intestine and reduced polyp growth through selective induction of apoptosis. Moreover, it interfered with polyp progression in Apc^{Min} mice, which best resembles the FAP (familial adenomatous polyposis, an autosomal dominantly inherited disease) phenotype, through modulating Wnt signaling. TQ reduced cell proliferation in the villi, down-regulated c-myc expression, and β-catenin translocation to the membrane in the polyps of Apc^{Min} mice [110].

TQ was found also to prevent fore-stomach carcinogenesis [61]. Moreover, TQ had chemosensitisation effect; it enhanced the 5-FU-induced killing of gastric cancer cells by modulating Bcl-2 and Bax expression and increasing the release of cytochrome c from the mitochondria. In addition the combined treatment of TQ with 5-FU represented a more effective anti-tumor manager than either agent alone in a xenograft tumor mouse model [111].

TQ had cytotoxic effect against pancreatic cancer and decreased viability in pancreatic cancer cells [112]. TQ inhibited the expression of Bcl-2, Bcl-xL, Mcl-1, survivin, and XIAP, and induced that of Bax. Furthermore, it suppressed COX-2 expression, PGE2 accumulation and NF-κB activation [112].

Moreover, a potential role has been reported for TQ as a chemopreventive agent at the early stage of skin tumourigenesis [113]. It induced cell-cycle arrest and apoptosis in neoplastic keratinocytes and squamous carcinoma cell lines A431, Hep2 and RPMI 2650 in both dose- and time-dependent manner, by inhibition of Akt and JNK phosphorylation [114]. Pretreatment of HR-1 hairless mouse skin with TQ attenuated 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of COX-2, and suppressed NF-κB signaling and the phosphorylation of Akt, JNK and the p38 MAP kinase. Besides, topical application of TQ induced the expression of HO-1 and GST in mouse skin. It also had inhibitory effects on the phosphorylation and degradation of IκBa, along with nuclear translocation and the DNA binding of NF-κB [115]. TQ inhibited migration and metastasis of both human and mouse melanoma cells, also *in vivo* in the B16F10 mouse melanoma model. It decreased the number of tumor nodules in lungs; this effect was accompanied by a decrease in expression of NLRP3 inflammasome resulting in reduction of proteolytic cleavage of caspase-1. Inactivation of caspase-1 by TQ resulted in inhibition of IL-1β and -18. Furthermore, treatment of melanoma cells with TQ blocked NF-κB activity [116]. Topical application of a TQ-rich fraction obtained from NS extract inhibited chemically induced mouse skin papillomagenesis [117]. TQ suppressed also 20-methylcholanthrene-induced fibrosarcoma tumorigenesis through inhibition of tumor incidence and of tumor burden [47].

TQ has been reported to prevent the proliferation and induce apoptosis in the human chronic myeloid leukemia KBM-5 cells by suppressing TNF-induced NF-κB expression [118]. It also degraded α and β tubulin of human astrocytoma cells in p53 deficient Jurkat cells [119]. TQ induced growth inhibition and apoptosis in some primary effusion lymphoma (PEL) cell lines. TQ treatment led to down-regulation of constitutive activation of AKT *via* generation of ROS, conformational changes in Bax protein, loss of mitochondrial membrane potential and release of cytochrome c into the cytoplasm, and caspase-dependent apoptosis. TQ-induced signaling caused caspase-9/3 activation and PARP cleavage and sensitized TRAIL-mediated apoptosis in PEL cell lines [120]. It also inhibited the proliferation of CD138+ cells isolated from multiple myeloma (MM) patient samples. Furthermore, in a xenograft mouse model with MM cell lines, it potentiated the apoptotic effects of bortezomib through modulation of various markers for survival and angiogenesis, such as Ki-67, VEGF, Bcl-2 and p65 expression [121]. TQ inhibited IL-6-induced Akt phosphorylation and IL-6-induced STAT3 expression. The effects of TQ on STAT3 phosphorylation correlated with the suppression of upstream protein tyrosine kinases JAK2 and c-Src. It also suppressed both inducible and constitutive STAT3 activation, which makes it a potentially effective suppressor of tumor cell survival, proliferation and angiogenesis [79].

TQ induced G2/M blockade in A549 human non-small cancer lung cells (NSCLC). Microtubule depolymerization induced by TQ was followed by apoptosis. In addition, it distorted spindle organization and suppressed tubulin polymerization by direct tubulin binding [122]. Thus, like other tubulin assembly inhibitors TQ can be classified as a microtubule-depolymerizing agent against cancers. TQ was able to induce apoptosis in both NSCLC and SCLC lung cancer cell lines, NCI-H460 and NCI-H146. It down-regulated NF-κB expression, and may be useful in overcoming cisplatin resistance resulted from overexpression of this factor. The combination of TQ and cisplatin was shown to be an active therapeutic combination against lung cancer both *in vitro* and *in vivo* [123].

Additionally, TQ exhibited chemosensitisation effects and sensitized pancreatic cancer cells to the chemotherapeutic drugs gemcitabine and oxaliplatin. TQ augmented anti-tumor activity of gemcitabine and oxaliplatin in pancreatic cancer (HPAC or BxPC3 cells) [112]. TQ (3 mg/mouse intragastric) alone or combined with either gemcitabine or oxaliplatin caused a substantial decrease in tumor weight [112]. TQ was effectively synergizing also with cisplatin also in lung cancer NCI-460 and NCI-146 cells [123].

TQ acted as an enhancer for the anti-cancer effect of DOX in HL-60 leukemia and 518A2 melanoma cell lines. It significantly increased the growth inhibition by DOX in HL-60 and multidrug-resistant MCF-7/Topo cells when TQ was added [124]. In mice, TQ (10 mg/kg per day) administered in drinking water improved the anti-tumor effect of ifosfamide and attenuated ifosfamide-induced Fanconi syndrome [125].

TQ also showed a powerful chemopreventive agent against fore-stomach tumor induced by B(a)P (a well known clastogen) and enhanced detoxification in mice [126]. Administration of TQ in drinking water one week before, during and after B(a)P treatment resulted in suppression of B(a)P-induced tumourigenesis. In this regard, anti-oxidant and anti-inflammatory activities and enhancement of detoxification processes were considered as the possible modes of TQ action against tumourigenesis [61]. TQ had anti-mutagenic and anti-clastogenic (anti-chromosomal damaging) activities [42,126] and was effective in inhibiting B(a)P-induced clastogenicity in mice. Daily intake of TQ (supplemented

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0.01% in drinking water for 28 days) after and before or during exposure to B(a)P significantly decreased the frequencies of chromosomal aberrations in bone marrow cells [126].

In spite of many types cytotoxic drugs having been developed for clinical applications, cancer chemotherapy is always accompanied with adverse effects, which may be serious in some cases. In addition, the cure rate of chemotherapy is limited through the development of resistance. As such, there is increasing interest in natural products to complement conventional medicine. The information reported above, alongside that reported elsewhere [127,128] indicates TQ as one of the most promising candidate drugs for the purpose of enhancing the anti-tumor potentials and/or reducing toxicity of chemotherapy.

1.4. Anti-microbial activities

In recent decades, the field of ethnobotanical research as a source for natural anti-microbial drugs and compounds has been expanding considerably. The usage of antibiotics and antibacterial chemotherapeutics is becoming less effective, because of resistance to them and side effects.

The anti-bacterial activity of TQ was reported against some bacterial strains, including *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Peudomonas aeruginosa*, and *Bacillus subtilis* [129]. TQ exhibited a significant bactericidal activity especially against Gram-positive cocci, and prevented cell adhesion to glass slides surface and avoided biofilm formation in human pathogenic strains. TQ supplementation was effective on the strong biofilm formers *S. aureus*, *Streptococcus salivarius* and *Streptococcus oralis*; the biofilm former of *Enterococcus faecalis*, *Gemella haemolysans*, *Pseudomonas aeruginosa* and *Streptococcus mitis* decreased but was not completely suppressed [130]. It has also been reported that TQ exhibits potent growth inhibitory against Gram-positive bacteria at low concentrations. In addition to reducing the number of adherent bacteria, TQ had an effect on the metabolic activity of cells embedded in biofilm. It efficiently killed staphylococci in suspension and prevented biofilms formation [131].

TQ possesses a selective anti-bacterial and resistance modifying activity against oral bacteria. The oral strains *S. aureus*, *Streptococcus mutans* and *S. salivarius* were sensitive to TQ with MIC values ranging from 8 to 64 μg/ml. It has been reported that TQ had synergistic effects in combination with anti-bacterial agents. By synergic effect, TQ reduced at least a 4-fold the tetracycline MICs value. In the case of benzalkonium chloride, an 8-fold reduction in MICs values was observed particularly for *S. aureus* and *Vibrio paraheamolyticus*. In addition, TQ inhibited the DAPI efflux activity, and the rate of DAPI accumulation in clinical isolates was enhanced with TQ [132].

The broth microdilution (BMD) method is widely used for determination of minimum inhibitory concentrations of anti-microbial agents. TQ vapors (32–512 μg/mL) caused the complete inhibition of staphylococcal growth in adjoining wells within a microtiter plate and this can significantly influence results of the standard BMD assay [133].

The epithelial cells pre-treated also the cells post-treated with TQ decreased the number of *Streptococcus pyogens* that were able to attach to the kidney epithelial cells *in vitro* in a dose dependent manner [134]. TQ treatment reduced sepsis-related morbidity and mortality in mice following LPS and live *E. coli*, and improved both renal and hepatic biomarker profiles. Moreover, mice treated with TQ exhibited lower levels of TNF-α and IL-2 [135]. This could be a strong candidate to protect against and/or treat this devastating condition for which no drug is suitably effective to date. Prostatitis plays a major role in mortality related to prostate diseases. TQ because of its anti-oxidant and anti-inflammatory effects improved acute bacterial prostatitis (ABP) induced by *P. aeruginosa*. ABP induced by *P. aeruginosa* had its oxidative effect on prostate tissue, and could regress following TQ administration. The levels of MDA and NO were found to be meaningfully lower in the groups given TQ [136]. TQ also was a protective and anti-proliferative against tissue injury resulted from prostatitis-induced by *E. coli*. Increases in MDA levels and histological damage caused by *E. coli* were improved considerably after TQ treatment, which particularly increased the activity of GSH-Px and reduced the activities of CAT and SOD [137]. Therefore, TQ could be a clinically valuable agent in the prevention of prostatitis caused by bacteria.

TQ may also be used as a potential therapeutic agent to normalize the dysregulated insulin production observed in highly active anti-retroviral therapy treated HIV-1 positive patients [138]. TQ possessed anti-tuberculosis activity against clinical isolates of *Mycobacterium tuberculosis* [139]. In addition, TQ exhibited anti-fungal activity against *Candida albicans*, *Candida tropicalis*, and *Candida krusei* [140] as well as the pathogenic dermatophyte strains *Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum gypseum* [141]. The ether extract of TQ (and to a lesser extent NS) markedly inhibited the growth of eight species of dermatophytes: four species of *Trichophyton rubrum* and one each of *Trichophyton interdigitale*, *T. mentagrophytes*, *Epidermophyton floccosum* and *M. canis* [142].

Treatment of TQ had a protective effect on schistosomiasis. Treatment of *Schistosoma mansoni*-infected mice with NS oil or TQ induced a protective effect on the infection-induced genotoxicity. This effect was evidenced by reduction in the percentage of chromosomal aberrations such as the numbers of chromosome deletions and tetraploidy induced by schistosomiasis [143]. Moreover, a virtuous anti-bacterial action against Paenibacillus larvae was observed by TQ (MIC values ranging from 8 to 16 mg/ml) [144].

These recommendations have made TQ a one of the preferred natural anti-microbial agent against many infections. However, since the exact mechanisms of the TQ anti-microbial activities are not clear, further works are required to investigate the main mechanisms by which TQ affect these organisms.

1.5. Hepatoprotective effects

In recent years, attention have been drawn to numerous plants and plant-derived compounds for the treatment of liver ailments. The protective effect of TQ on liver injures have been demonstrated by some studies. TQ could neutralize the negative influences of various damaging agents on liver tissue. Oral administration of TQ (100 mg/kg) resulted in substantial protection against the hepatotoxicity of carbon tetrachloride (CCl4). TQ inhibited the non-enzymatic lipid peroxidation in liver homogenates and this suggests that the protective action of TQ against CCl4-induced toxicity may be mediated through the anti-oxidant properties of TQ. The anti-oxidative nature of TQ as an inhibitor of lipid peroxidation in liver compares with that of butylated hydroxytoluene (BHT) as a standard anti-oxidant [24]. TQ protected isolated rat hepatocytes in suspension culture against tert-butylhydroperoxide (TBHP)-induced toxicity. It prevented depletion of intracellular glutathione, and maintained the integrity of cell membranes as evidenced by decreasing leakage of ALT and aspartate aminotransferase (AST), as well trypan blue uptake. This protective role of TQ is comparable with that of silybin, a known hepatoprotective agent possessing anti-oxidant properties [43]. TQ oral injection efficiently reduced acetaminophen-induced hepatotoxicity, as evidenced by decreased serum ALT activities. This positive effect is possibly through increase resistance to oxidative and nitrosative stress and its ability to enhance GST as well the mitochondrial energy (ATP) production. TQ produced less nitrate/nitrite than acetaminophen-treated mice and it inhibited nitric oxide production [56]. On the other side, TQ supplementation induced quinone reductase and glutathione transferase in liver; thus, it may increase resistance

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to acetaminophen-induced toxicity through induction of phase-2 enzymes [27]. Lipid peroxide accumulation and reduction of GSH content, and GST and DT-diaphorase activities were observed in the liver of B(a)P-treated tumor-bearing mice. Mice treated with TQ along with B(a)P showed normal hepatic lipid peroxides, and normal enzyme content and activities compared to the control group [61].

TQ showed positive effects against cadmium-induced hepatotoxicity in mice. Pre-exposure with TQ prior to cadmium exposure decreased oxidative protein damage; it increased the expression of SOD, and prevented the reduction of CAT when exposed to cadmium. There was also an elevation in GSH and non-protein thiol (NP-SH) levels after TQ exposure [145]. TQ also ameliorated liver dysfunction induced by cyclophosphamide in rats through up-regulation of anti-oxidant mechanisms [55]. TQ considerably inhibited tamoxifen-induced hepatic GSH depletion. Consistently, it normalized the activity of SOD, inhibited the rise in TNF-α and ameliorated the histopathological changes; and in conclusion, it protected against tamoxifen-induced hepatotoxicity in female rats [146].

Treatment with TQ was protective against cypermethrin-induced hepatotoxicity in mice, as proved by a decrease in serum AST and ALT activity. It also exhibited protective action against the cypermethrin-induced necrosis of hepatic cells, with degeneration, dilatation of sinusoids and dissociated remark cordons in livers of mice. Moreover, it induced cell proliferation leading to enhanced regeneration after tissue damage [147]. TQ is a promising agent in maintenance of normal hepatic function during treatment with anti-tuberculosis drugs and had therapeutic effect against anti-tuberculosis drugs-induced liver damage. TQ administration for 8 weeks (3 days/week) attenuated the increases in the levels of hepatic AST, ALT and ALP enzymes, and caused a consequent recovery toward normalization indicating stabilization of plasma membrane as well as repair of hepatic tissues [148]. TQ was protective against aflatoxin B1 (AFB1)-induced hepatotoxicity. The AST, ALT, ALP and MDA levels were significantly lower in groups that received TQ compared with AFB1, and liver sections of AFB1 intoxicated mice showed necrosis and degradation while treatment with TQ helped to normalize liver architecture [57]. Solid lipid nanoparticles (SLNs) of TQ acted against paracetamol-induced liver cirrhosis and fibrosis [13]. TQ remarkably reduced thioacetamide-induced liver fibrosis and inflammation. This effect accompanied by reduced α-smooth muscle actin (α-SMA) protein and mRNA expression, collagen- and tissue inhibitor of metalloproteinase-1 (TIMP-1). Moreover, TQ down-regulated the expression of toll-like receptor 4 (TLR4) and decreased pro-inflammatory cytokine levels. It also inhibited PI3K phosphorylation, enhanced the phosphorylation AMPK and liver kinase B (LKB)-1 [149].

TQ has been shown to possess anti-oxidant and anti-inflammatory effects both *in vitro* and *in vivo*, and these roles are directly connected to hepatoprotection. It may reduce oxidative stress through direct anti-oxidant effect as well as induction of endogenous anti-oxidant enzymes leading to the prevention of liver toxicity. TQ improved liver anti-oxidant capacity and enhanced the expression of liver anti-oxidant genes in hypercholesterolemic rats. It increased the expression of SOD1, CAT, GSH-Px genes and elevated hepatic SOD and GSH-Px levels [50]. Administration of TQ was effective in increasing the activities of quinone reductase [27,47] and glutathione transferase [27], which makes it a promising manager against chemical carcinogenesis and toxicity in liver. It was also reported that the oral administration of TQ in bile duct ligated rats, reduced liver oxidative damage and ductular proliferation [82].

TQ had protective role against chromosomal aberrations induced by schistosomiasis infection in mice. Spleen and bone marrow cells in both *in vitro* and *in vivo* experiments were used to evaluate the potentially positive activities of TQ on the induction of chromosomal aberrations [143]. Oral TQ treatment greatly reduced liver injury and tumor markers expression, prevented hepatic nodule formation and reduced tumor multiplicity in NDEA-induced hepatic cancer bearing rats. It decreased the damaging alterations by abrogating cell proliferation, by strongly inducing G1/S arrest in cell cycle, and thus exhibited an advantageous role in the treatment of hepatocellular carcinoma [150].

TQ caused also concentration dependent genotoxicity in hepatocyte primary cultures, and thus it induced chromosomal aberrations and micronucleated cells [151].

In general, these studies showed that TQ may be a potential candidate for the therapy of hepatic fibrosis, carcinogenesis and inflammation, although its use is potentially associated with health risk [151]; thus further researches are needed to determine the effects of TQ on the liver enzymes and histology alternations and its risk to benefit ratio.

1.6. Hypoglycemic and anti-diabetic effects

Diabetes mellitus is usually associated with many problems. Diabetes in humans induces chronic complications such as cardiovascular damage, nephropathy and neuropathy. A scientific investigation of traditional plant remedies for diabetes may lead for the development of alternative drugs and therapeutic strategies. More than 1200 species of plants reported to have been used to treat diabetes and/or investigated for anti-diabetic activities, including *N. sativa* [152].

TQ has anti-diabetic activities apart from possessing many other valuable pharmacological actions. The most common model of human diabetes is the streptozotocin (STZ)-induced diabetes in animal models. TQ exerted anti-hyperglycemic effects and ameliorated blood glucose levels in gestational diabetes mellitus. Daily gastric administration of TQ (50 mg/kg for 30 days) to STZ-induced diabetic hamsters efficiently reduced both fasting blood glucose and glycated hemoglobin levels, and reduced the gluconeogenesis observed under the diabetic condition. Glucose production was considerably lower in hamsters treated with TQ, since the glucose-lowering effect of TQ is not related directly to insulin action; also, it decreased gluconeogenesis by suppressing the synthesis of gluconeogenic enzymes [153]. TQ improved the glycemic status in STZ-nicotinamide-induced diabetic rats. Oral administration of TQ for 6 weeks resulted in a major decrease in plasma glucose and an increase in insulin levels. TQ normalized the perturbed carbohydrate metabolism by enhancing glucose utilization and by decreasing hepatic glucose production. TQ at 80 mg/kg dose decreased the activities of the gluconeogenic enzymes glucose-6-phosphtse and fructose-1,6-bisphosphatase [25]. Treatment of rats with TQ decreased the diabetes-induced increases in tissue MDA and serum glucose, and increased serum insulin and tissue SOD. TQ improved most of the toxic effects of STZ, including DNA damage, mitochondrial vacuolization and fragmentation, and preserved beta-cell integrity by decreasing oxidative stress. Thus, the anti-diabetic action of TQ could be due, in part, to amelioration of the cellular and subcellular structures of beta-cells [64]. Treatment of STZ-diabetic rats with TQ blocked the expression of COX-2 enzyme, lipid peroxidation and MDA levels, along with increased the level of SOD in the pancreatic tissue [154].

Nutritional supplementation of gestational diabetes mothers with TQ during pregnancy and lactation periods improved diabetic complications and maintained an efficient T cell immune response in their offspring, providing a protection in later life. The restorative effect of TQ on IL-2 level and T-cell proliferation, and the following rescue of both circulating and thymus homing T cells in the offspring of diabetic mothers enhanced the immune response [155]. Similarly, the data indicated a decrease in the percentage of

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abortions, an increase in the number of successful pregnancies and an improvement of mortality among new pups born to diabetic mothers. TQ improved aberrant hydroperoxide and ROS levels in pups and these results may be mediated by increase in the levels of GST, GSH and CAT, and a decrease in DNA damage [156].

Accumulation of advanced glycation end-products in tissues and serum plays important role in diabetes-associated complications. TQ had anti-glycating effect and reduced diabetic problems due to protein glycation [157]. It regulated the plasma concentrations of cholesterol and triglycerides; plasma triglyceride and cholesterol levels diminished significantly in the diabetic rats treated with TQ [158]. Orally administration of TQ (10 mg/rat/day) affected experimental diabetic neuropathy. TQ has been associated with recovery of the histopathologic changes in sciatic nerves, and myelin breakdown decreased meaningfully after treatment with TQ [159].

Oxidative stress and inflammation play a crucial role in the progression of diabetic problems. Inhibition of adenosine kinase by ABT702, an adenosine kinase inhibitor, decreased extracellular adenosine levels to moderate renal injury in STZ-induced diabetes. In fact, the nephroprotective effects of ABT702 could be attributed to the reduction in renal inflammation and oxidative stress in diabetic mice [161]. STZ-induced diabetes caused nephropathy, and TQ therapy resulted in renal morphologic and functional improvement [160]. The anti-oxidant activity of TQ may relieve damage to beta-cells caused by STZ. TQ was effective for beta-cell protection against damage, through the down-regulation of inflammatory activity mediated by NO pathway [22]. TQ administered intraperitoneally in diabetic rats at 3 mg/kg normalized the elevated levels of the pro-inflammatory cytokines IL-1β and TNF-α [22]. STZ-diabetes induce an increase in heart and brain NO and MDA concentrations, changes that were mitigated by post-treatment of rats with TQ. STZ-diabetes induced a decrease in GST, GSH and CAT, and these lowered levels were improved by TQ administration. Serum cardiac creatine kinase muscle and brain types (CK-MB) was decreased in the diabetic rats, which recovered with TQ administration. During diabetes, there was a marked increase in norepinephrine and dopamine and a marked decrease in serotonin level; these were partly reversed by TQ orally [162].

These findings provide scientific bases to the widespread use of NS seeds as an anti-diabetic remedy in Middle East folk medicine [163]; still the information on TQ action is still insufficient especially as far as efficacy on diabetic complications is concerned.

1.7. Gastroprotective effects

Ample evidence exists on the protective effect of TQ on gastrointestinal tract. TQ possessed protecting effect against gastric lesions, which may be related to the protection of the gastric mucosal redox state. Anti-oxidant property of TQ corrected ischemia/reperfusion (I/R)-induced gastric dysfunction and stomach ulcer in rats. It increased GSH level and SOD activity, and reduced MDA content and MPO activity [164]. Gastric I/R-mediated alteration in acid concentration, acid output and pepsin was markedly hindered by pre-treatment with TQ. The gastroprotective effects of TQ resulted from inhibition of the proton pump (H^+/K^+-ATPase), acid secretion and neutrophil infiltration, though improving mucin secretion and content, and NO production. By reducing gastric oxidative injury, TQ combated the I/R-induced lipid peroxidation, and prevented depletion of GSH and SOD. All these mechanisms maintained normal gastric mucosal barrier integrity. In addition, TQ corrected the altered parameters in a comparable manner to that of the reference drug used, omeprazole and combination of it with omeprazole was synergic [165]. The administration of TQ on the acetic acid-induced colitis by intracolonic injection of 3% acetic acid showed that pretreatment of animals for 3 days with 10 mg/kg TQ led to complete protection against colitis, with a similar or even higher effect than sulfasalazine, an anti-colitis drug [80]. Likewise, TQ protected against the ulcerating effect of alcohol and diminished most of the biochemical adverse effects induced by alcohol in gastric mucosa. It affected CAT activity in gastric tissue [48]. It attenuated acetic acid-induced colitis as evidenced by the blinted release of histamine, to which the prevention of GSH depletion and lipid peroxidation may have also helped [80]. In addition, an anti-histaminic effect is an important defensive mechanism against gastric injury [166] and TQ has a potent anti-histaminic effect [80].

TQ had a helpful role in indomethacin-induced gastric injury model and was able to decrease acidity in indomethacin-induced gastric ulcer models [167]. TQ treatment was also able to reduce the number of macrophages and the gravity of gastric mucosal lesions, and it was effective in controlling bacterial translocation and improving intestinal barrier function in rats [168].

Altogether, these studies confirm the gastroprotective effects of TQ in animal models and this makes it possible to use TQ as a natural drug against gastrointestinal defects in human.

1.8. Neuroprotective effects

TQ has demonstrated several beneficial neuropharmacological properties. Administration of TQ powerfully induced protection in cultured rat primary hippocampal and human induced pluripotent stem cell (hiPSC)-derived neurons cells against α-synuclein-induced synaptic toxicity [169]. TQ protected against MPP+- and rotenone-induced cell death in primary dopaminergic neurons in cell cultures obtained from patients with Parkinson's disease. It sheltered cultured dopaminergic TH immunoreactive cells from degeneration induced by MPP+ and rotenone toxicities; and the anti-oxidant properties of TQ appeared to play an important role in this respect [170]. TQ also inhibited oxidative stress and neuropathy in STZ-induced diabetic rats. It reduced norepinephrine and dopamine and enhanced serotonin levels [162]. TQ suppressed morphine-induced brain oxidative stress, and development of morphine tolerance and dependence in mice. The elevation in brain glutamate level and the increase in the expression of brain iNOS and NO overproduction by morphine can be reduced by TQ [171]. TQ has helpful properties against neurotoxic effects of lead (Pb) (a ubiquitous heavy metal) in rats. Co-treatment of lead-exposed rats with TQ distinctly reduced the incidence of lead-induced brain lesions [172]. TQ by anti-oxidant properties protected brain tissue of irradiated rats from radiation-induced nitrosative damage [65]. TQ had a protective role against ethanol-induced neuronal apoptosis in primary rat cortical neurons by decreasing ethanol-mediated mitochondria-dependent apoptosis [173]. It caused morphologic improvement and prevented neurodegeneration in the hippocampus after chronic toluene exposure, and the distorted nerve cells were mostly absent in the TQ-treated rats [33].

Beta-amyloid (Aβ) peptides are considered to play a main role in the pathogenesis of Alzheimer's disease (AD) and compounds that can prevent pathways of Aβ-induced neurotoxicity may be potential therapeutic agents for treatment of AD. TQ protected against Aβ-induced toxicity and the impairment of synaptic function, as well as decreased αβ aggregation in primary hippocampal and cortical neurons. Treatment with TQ inhibited β-amyloid peptide 1–42 sequence Aβ(1–42)-induced neurotoxicity, and efficiently attenuated Aβ(1–42)-induced mitochondrial membrane potential collapse in cultured hippocampal neurons, also suppressing ROS generation caused by Aβ(1–42) [174]. TQ also abridged Aβ-induced toxicity through inhibition of mitochondrial dysfunction and oxidative stress in PC12 cells (derived from a pheochromocytoma of a rat adrenal medulla); moreover it protected rats against transient forebrain ischemia-induced damage in the hippocampus [34,173]. A significant increase in thiobarbituric acid reactive substances (TBARS) content, NO level and activity of

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acetylcholine esterase was observed in Aβ exposure, which was normalized by TQ pre-treatment. Furthermore, TQ could reduce neuronal damage and loss through counteracting oxidative stress, *via* ameliorating glutathione and its dependent enzymes (GSH-Px, glutathione reductase) which are depleted by Aβ(25–35) [175]. TQ has the potential to reduce Aβ(1–40)-induced neuronal cell death in primary cultured cerebellar granule neurons (CGNs). Pre-treatment of CGNs with TQ and subsequent exposure to Aβ(1–40) protected CGNs against the neurotoxic effects of the latter. In addition, CGNs were better preserved with intact cell bodies, extensive neurite networks, a loss of condensed chromatin and less free radical generation than those exposed to Aβ(1–40) alone [176].

I/R injury resulting from stroke leads to metabolic distress, oxidative stress and neuroinflammation, making it likely that therapeutic intervention strategy needed which affect oxidative stress and inflammation [177]. TQ was effective against transient forebrain ischemia-induced damage in the rat hippocampus. TQ administration (5 mg/kg/day orally) 5 days before ischemia continued during the reperfusion time attenuated forebrain ischemia-induced neuronal damage, as revealed by reduction in the number of dead hippocampal neuronal cells. Pretreatment of ischemic rats with TQ decreased the elevated levels of MDA, and improved GSH content, CAT and SOD activities to normal levels [83].

In traditional medicine, NS was known as an anti-convulsant and some studies also have shown anti-convulsant effects of NS and TQ. Hosseinzadeh and Parvardeh [23] investigated the anti-convulsant activity of TQ, using a single dose of pentylenetetrazol (90 mg/kg) as petit mal epilepsy model in mice. This anti-convulsant activity was through an opioid receptor-mediated improvement in GABAergic tone. The anti-nociceptive effect of morphine was increased in TQ-pretreated mice. TQ produced anti-nociceptive effects mostly through the supraspinal opioid systems, mainly μ- and κ-opioid receptor subtypes [20]. TQ was beneficial for lessening neuropathic pain following spinal cord injury (SCI) and it had analgesic/anti-nociceptive effects on central pain following SCI. Also total oxidant status, NO, MDA, IL-1β, and TNF-α levels were lower in the TQ groups than in the control group [178]. TQ showed anti-anxiety activity through GABAergic and nitriergic modulation. TQ (10 and 20 mg/kg) produced considerable anti-anxiety effects in unstressed mice without altering nitrite levels, but only the higher dose (20 mg/kg) of TQ augmented the GABA content in unstressed mice. In stressed mice, TQ (20 mg/kg) showed anxiolytic effects, with a decrease in plasma nitrite and reversal of the reduced brain GABA content [81]. Additionally, TQ demonstrated anti-depressant effects. It further demonstrated anti-oxidant effects by reducing TBARS and increasing reduced GSH levels. It may be suggested that TQ is a potential candidate for the management of depression [179]. A double-blinded placebo controlled randomized trial was done where TQ (dose of 1 mg/kg) was administered as an adjunctive therapy to 22 children with refractory epilepsy, and its effects on frequency of seizures were compared with those of a placebo. The patients were assigned in two groups and received either TQ or placebo for a period of four weeks, and then, during the two weeks of wash out period, they received only their preexisting anti-epileptic drugs; after cross-overing, they received TQ or placebo for a period of four weeks again. From this study, it concluded that TQ had anti-epileptic effects [180].

TQ showed a very pleotropic favorable effect on experimental allergic EAE [76] and suppressed NF-κB activation in brain and spinal cord of EAE [76].

These results indicate TQ as a promising tool, if properly used, in the prevention and treatment of a variety of nerves disorders such as ischemia-reperfusion injury, depression, seizure, Alzheimer and Parkinson's disease.

1.9. TQ and cardiovascular problems

Cardiovascular diseases remain one of the leading causes of death worldwide. Several lines of evidence suggest that TQ is a therapeutic option in cardiovascular complications. Doxorubicin (DOX) has a wide spectrum of anti-tumor activity along with cardiotoxicity as a major side effect. Pretreatment with TQ protected against DOX-induced cardiotoxicity without compromising its anti-tumor activity [26,41]. TQ (8 mg/kg/day) administered with drinking water starting 5 days before a single injection of DOX and continued during the experimental period improved the DOX-induced heart toxicity in mice. This finding was supported by significant declines in serum LDH and creatine kinase elevated levels, and by histopathological improvement of cardiac tissue [26]. TQ acted as an inhibitor of lipid peroxidation and superoxide radical scavenger in DOX-induced cardiotoxicity in rats [41]. Cyclophosphamide increases serum creatine kinase, creatinine, urea, LDH, cholesterol, triglycerides and TNF-α. In heart tissue, it also increases TBARS and total nitrate/nitrite, and reduces GSH-Px, CAT and ATP levels. Supplementation by TQ resulted in a complete reversal of all these changes to their control values [58]. Moreover, TQ exhibited protection against the cypermethrin-induced necrosis, degeneration, and loss of striation in heart; it resulted in reversal of cypermethrin-induced oxidative stress and lipid peroxidation [147].

The state of hyperhomocysteinemia (HHcy) appears to be accompanied by high risk of coronary, cerebral and peripheral vascular disease; and the pathogenesis of HHcy is known to be linked with free radical formation. Pretreatment of rats with a dose of 100 mg/kg TQ orally, for one week almost entirely protected against methionine-induced HHcy. Consequently, utilizing TQ against the cardionegative impacts of HHcy may be valued [35]. Intravenous administration of TQ to rats decreased the arterial blood pressure and the heart rate in a dose-dependent manner [21]. It was effective in protection of rats against N-nitro-L-arginine methyl ester (L-NAME)-induced hypertension, perhaps *via* anti-oxidant action [181]. Treatment with TQ decreased the elevated creatinine, and increased GSH levels compared to normal levels, and inhibited the production of superoxide radicals in enzymatic and non-enzymatic systems. It reduced the increase in systolic blood pressure induced by L-NAME in a dose-dependent manner [181].

Pretreatment with TQ markedly prevented diesel exhaust particles-induced pulmonary and cardiovascular changes. Pretreatment of mice with TQ prohibited diesel exhaust particles-induced decrease of systolic blood pressure, increased SOD activity and decreased IL-6 level. It also stopped the decline in platelet numbers [182].

1.10. TQ against respiratory diseases

The therapeutic effects of the NS seeds on respiratory problems including asthma and dyspnea have been described in Iranian ancient medical books [183]. TQ can neutralize the negative results of some injuring agents. TQ showed anti-apoptotic effect and attenuated lung injury induced by chronic toluene exposure in rats [36]. It also alleviated the progression of pulmonary fibrosis that induced by bleomycin in rats. TQ counteracted emphysema in air alveoli, inflammatory cell infiltration, lymphoid hyperplastic cells activation surrounding the bronchioles, and the overexpression of activated form of NF-κB in lung tissue induced by bleomycin. It also restored anti-oxidant enzyme activity of SOD and GST toward normal values [60]. TQ was protective against cyclophosphamide-induced pulmonary damage. Cyclophosphamide increased the level of serum biomarkers total protein, LDH and TNF-α. Treatment of rats with TQ 7 days before and after cyclophosphamide injection reduced the alterations in lung and serum biomarkers associated

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with inflammatory reactions, with less lipid peroxidation and restoration of anti-oxidants. It also improved cyclophosphamide-induced histopathological changes in lung tissue [59]. In addition, TQ relaxed pulmonary arterial rings and caused a concentration-dependent decrease in the tension of the pulmonary arterial rings precontracted by phenylephrine. This relaxant effect may be due to activation of ATP-sensitive potassium channels and probably by non-competitive blocking of serotonin, alpha1 and endothelin receptors [184].

TQ had potential anti-inflammatory role during the allergic response in the lung. Intraperitoneal injection of TQ before airway challenge of OVA-sensitized mice attenuated allergic airway inflammation by preventing Th2 cytokines and eosinophil infiltration and goblet cell hyperplasia in the airways, both *in vivo* and *in vitro*. TQ showed a significant effect in inhibition of IL-4, -5 and -13, and in induction of IFN-γ production in the BAL fluid [21]. TQ had an anti-inflammatory action during the allergic response in the lung through the inhibition of Th2-driven immune response. Injection of TQ intraperitoneally for 5 days before the first OVA challenge diminished airway inflammation, and this attenuation of inflammation was associated to the inhibition of COX-2 protein expression and PGD2 production [70].

Chronic airway inflammation is a key feature of bronchial asthma and leukotrienes are potent inflammatory mediators that play a role in the pathophysiology of asthma. Administration of TQ before OVA challenge inhibited expression by lung cells of 5-lipoxygenase, the main enzyme in leukotriene biosynthesis, and moderated the levels of LTB4 and LTC4. This was accompanied by a decrease in Th2 cytokines, BAL fluid and lung tissue eosinophilia [69]. It also caused a concentration-dependent decrease in the tension of the tracheal smooth muscle precontracted by carbachol in the guinea pig. TQ induced relaxation of guinea pig's isolated trachea, mediated by inhibition of lipoxygenase products of arachidonic acid metabolism and by non-selective blocking of the histamine and serotonin receptors [185].

Furthermore, TQ has been shown to be useful for treatment of acute respiratory distress syndrome in a rat model [186]. Intravenous administration of TQ induced rises in the intratracheal pressure, without any effect in the respiratory rate in urethane-anaesthetized guinea pig [19]. These results from TQ demonstrate its value in respiratory disorders, and further support the traditional use of black seeds to treat respiratory complaints such as bronchial asthma.

1.11. Nephroprotective effects

Reports have shown protective effects of TQ on kidneys in various pathogenic conditions. TQ provided an improvement in renal lesions resulted from various toxic agents because it showed a defensive effect, in a part by attenuating oxidative stress and inflammation. TQ supplementation prevented gentamicin-induced acute renal failure in rats by improving mitochondrial function and augmenting ATP production. It reduced the nephrotoxicity indexes and degenerative changes induced by gentamicin. It also reversed the gentamicin-induced increase in blood urea nitrogen, creatinine, and TBARS, which is accompanied with the rise in the total anti-oxidant status in renal cortex, including GSH level, GSH-Px and CAT activities [52]. TQ protected against mercuric chloride ($HgCl_2$)-induced renal damage in rats. The decline of anti-oxidant enzymes, increase of serum creatinine, proliferative response and histological damage caused by $HgCl_2$ are ameliorated by TQ usage [54]. TQ, when administered in drinking water (50 mg/l), improved the cisplatin-induced acute kidney damage, as well developed cisplatin therapeutic effects on rodent models. This was marked by significant reduction in serum urea and creatinine, and improvement in polyuria, kidney weight, and creatinine clearance [30]. It was also protective against vancomycin-induced kidney injuries. The levels of serum blood urea nitrogen, creatinine and MDA were increased in the vancomycin group, and activities of SOD and GSH-Px in kidney tissue were reduced. TQ administration ameliorated noticeably these changes [53]. Administration of TQ caused renal morphologic and functional improvement after STZ-induced diabetes in rats [160]. TQ in the drinking water (5 mg/kg per day) before and during ifosfamide treatment improved the severity of ifosfamide-induced renal damage. It significantly amended ifosfamide-induced phosphaturia, glucosuria, elevated serum creatinine and urea, and normalized creatinine clearance rate. Moreover, it prevented renal GSH depletion and lipid peroxide accumulation *via* anti-oxidant mechanisms [125]. Furthermore, TQ had positive effects on DOX-induced hyperlipidemic nephropathy in rats and decreased serum triglycerides and cholesterol. Treatment with TQ (10 mg/kg/day) repressed DOX-induced proteinuria and albuminuria, also lowered total triglycerides, total cholesterol and lipid peroxidation in the kidneys. Moreover, non-protein sulfhydryl (NPSH) content and CAT activity in the kidneys of TQ-treated DOX group were markedly elevated compared with DOX alone [51]. DOX-induced nephrosis involved a redox imbalance in renal tissue; this was reversed by TQ. Animals treated with TQ showed a reduced renal damage with normalization of the elevated levels of serum urea, creatinine and urinary albumin excretion, as equivalents of MDA, accompanied by substantial increases in the activities of SOD and GST, along with reduction of the renal oxidase NOX-4 level [187].

TQ also was effective in hepatorenal dysfunction induced by renal I/R. Renal I/R resulted in an increase in MDA level and reduction in GST and SOD activity in liver and kidney tissues, and TQ treatment caused the reversal of these changes. It reduced spermidine/spermine N-1-acetyl-transferase (SSAT), a catabolic enzyme that participates in polyamine metabolism, and CYP3A1 gene expression in liver and noticeably in kidney of rats [188]. TQ administration protected the kidneys from oxidative damage caused by pyelonephritis. In the pyelonephritis, SOD and CAT activity, and MDA levels were significantly abnormal; and levels of these factors repaired in the pyelonephritis treated by TQ [189]. Supplementation of TQ exhibited protective action against the cypermethrin-induced-sloughing off epithelial cell, shrinkage of glomeruli, and necrosis of renal tubules in kidneys of mice [147]. These data suggest that TQ can be applicable as a protective agent for nephropathies.

1.12. Effects of TQ on bone and joint complications

The effects of TQ on bone metabolism, bone formation and bone healing as well disorders related to bone was reported in some studies. TQ may be advantageous in osteogenesis. Kirui et al. [190] examined the physiological responses of TQ in the femoral defect animal model and showed sustained delivery of drug was effective in bone healing. It has shown that the alveolar bone loss due to periodontitis was reduced by gastric TQ given to rats. TQ also reduced the number of osteoclasts and raised osteoblastic activity [191]. TQ had anabolic effects and induced the proliferation and the mineralization of MC3T3-E1 osteoblast cells. It induced the expression of differentiation related genes including ALP (an early marker for mature osteoblasts), osteocalcin and osteopontin (phenotypic markers for the later stage of osteoblast differentiation); it also increased the expression of BMP-2, and up-regulated the phosphorylation of ERK signaling pathway and activated MAPK pathway. TQ's effect on cell maturation was accompanied by an increase in BMP-2 expression and activation of the ERK pathway. The ability of TQ to enhance the expression levels of early, intermediate and late differentiation markers indicates that it can be used for interference at various levels from early to terminal stage of differentiation process [192]. In addition, systemic use of TQ in rats

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resulted in measurable acceleration of bone formation. Systemic administration of 10 mg/kg of TQ can promote bone formation and may be beneficial in prevention of relapse following the rapid maxillary expansion (RME) procedure. In light of potent anti-oxidant properties, TQ may prove to have an imperative role in accelerating bone formation and in the shortening of the retention period involved in RME. TQ reduced the ROS production and the level of pro-inflammatory cytokines IL-1α and -6 and TNF-α, cytokines that can lead to differentiation of osteoclast precursors and osteoclast activity to cause bone resorption [193].

A link between inflammation and bone homeostasis has been attributed to the effects of IL-1β, IL-6, TNF-α, IFN-γ and PGE2 that are abundantly expressed in patients with RA and in the arthritic joints of rat with collagen-induced arthritis. Blockade of these resulted in a reduction of disease severity and bone resorption [194]. Treatment with TQ shifted the balance of cytokines toward a bone protecting pattern that acted to both decrease levels of TNF-α, IL-1β, IFN-γ and IL-6, and raise the levels of IL-10 [72] a potent anti-inflammatory cytokine that limits cartilage and bone pathology in RA [195]. From these results, TQ appears to have cartilage/bone protective effects mediated through the inhibition of pro-inflammatory and induction of anti-inflammatory mediators. TQ prominently abolished also LPS-induced IL-1β, TNF-α, MMP-13, COX-2, and PGE2 induction in isolated RA fibroblast-like synoviocytes. Furthermore, LPS-induced phosphorylation of p38 MAP kinase and NF-κB-p65 were also blunted by TQ. The oral administration of 5 mg/kg/day TQ in a rat adjuvant-induced arthritis model of RA significantly decreased the serum levels of H2O2-induced 4-hydroxynonenal, and increased bone turnover markers, such as ALP and tartrate-resistant acid phosphatase. The protective effects of TQ against articular diseases were also manifest from the reduction in arthritis scoring and bone resorption [75].

1.13. Effects of TQ on reproductive system

Acute bacterial prostatitis (ABP) induced by *P. aeruginosa*, which has an oxidative effect on prostate tissue, regressed following TQ administration as shown by biochemical and histological results. In the ABP groups given TQ, the levels of MDA, SOD and NO, and the GSH-Px activity were found to be normal compared to control group, indicating an antioxidant activity of TQ [136]. It also had a protective effect against tissue injury in ABP-induced by *E. coli*. TQ reduced MDA, and improved the activities of GSH-Px, CAT and SOD [137]. TQ, through its anti-oxidant and anti-inflammatory activities, protected the testes against the injurious effect of cadmium exposure. It decreased the cadmium-induced reductions in serum testosterone, elevated testicular glutathione and SOD activity, also decreased the elevations of testicular MDA, NO and cadmium ion levels resulting from cadmium chloride administration. In addition, the cadmium-induced expression of iNOS, TNF-α, COX-2 and NF-κB was diminished by TQ in testicular tissue [196].

TQ had favorable effect against lead (Pb)-induced inhibition of rat testicular functions. It protected against Pb-induced impairment of testicular steroidogenic and spermatogenic functions. When co-administrated with lead, TQ significantly improved the low plasma testosterone level and the decreased epididymal sperm count caused by lead [197]. TQ treatment declined interstitial space dilatation in methotrexate testicular injuries in mouse model; it reversed histological alterations, augmented total anti-oxidant capacity and blocked the increase in the MPO activity, which occurred in methotrexate-treated group [198]. TQ was cytotoxic toward breast cancer [95,96], ovarian adenocarcinoma [93], cervical squamous carcinoma [99], and prostate cancer [100,101].

In conclusion, considering these data, the use of TQ could be a viable option for reproductive system diseases.

1.14. Hypolipidemic effects

TQ was capable of lowering plasma cholesterol level in animals, in part, because of its anti-oxidant activity. It attenuated hepatic oxidative stress induced by high cholesterol diet in rabbits [196]. It also had protective effect on development of atherosclerosis in cholesterol-fed rabbits. Administration of TQ with cholesterol-enriched diet reduced total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides and TBARS concentrations, while increased high-density lipoprotein (HDL)-cholesterol as well as glutathione content compared to control group [200].

TQ supplementation along with ethanol and high fat diet markedly decreased the levels of serum lipase, amylase and caspase-1. A dose of 100 mg/kg body weight was found to provide optimal effect on pancreas against high fat diet-induced pathological changes [201]. In addition, TQ generated a hypocholesterolemic effect in diabetic rats through regulation of cholesterol in two main mechanisms, firstly by increasing uptake of LDL-cholesterol *via* up-regulation of hepatic LDL receptor gene and, secondly, by suppressing the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) gene [158]. It also prevented cardiovascular disease risks and parameters *via* a reduction in HMG-CoAR, along with an increase in arylesterase activity. TQ may thus be used in the protection of hyperlipidemia and atherosclerotic problems [202]. It had positive effects on DOX-induced hyperlipidemic nephropathy in rats and could decrease serum triglycerides and cholesterol, as shown by the reduction in total triglycerides, total cholesterol and lipid peroxidation in the kidneys after treatment with TQ (10 mg/kg/day) [51]. The results, taken together support a role for TQ in the treatment of hyperlipidemia/dyslipidemia and associated complications.

1.15. Anti-histaminic effects

Histamine is released by basophils and mast cells, producing allergic reactions. Some of anti-inflammatory effects of TQ have been related to its role in inhibition of histamine production and/or release. It reduced the ocular symptoms in allergic conjunctivitis by attenuating histamine in mice exposed to OVA [71]. TQ induced relaxation of guinea pig isolated trachea, which is mediated by non-selective blockade of the histamine receptors. It abolished the effects of histamine and serotonin on the tracheal and ileum smooth muscles [185]. Moreover, TQ protected against acetic acid-induced colitis in rats by its ability to inhibit the release of the mediators platelet-activating factor (PAF) and histamine [80], and it is proven that a main mechanism by which TQ acts against gastric injury is an anti-histaminic action [166].

1.16. TQ and fibrosis

TQ has been investigated for its anti-oxidant and anti-inflammatory activities in some models since its isolation in 1960s; however, its possible anti-fibrotic effect is yet not very clear.

TQ attenuated liver fibrosis through hindering PI3K and down-regulating TLR4 signaling pathways in activated rat hepatic stellate cell line, T-HSC/Cl-6. It induced apoptosis, reduced the expression of CD14 and TLR4, and suppressed the expression of collagen-I and PI3K, and Akt phosphorylation. Furthermore, the levels of serum ALT and AST decreased by TQ [203]. TQ substantially attenuated thioacetamide-induced liver fibrosis and inflammation. It down-regulated the expression of TLR4, inhibited PI3K phosphorylation, activated LKB1-AMPK signaling pathway, and decreased extracellular matrix accumulation [149]. Solid lipid nanoparticles (SLNs) formulation of TQ inhibited paracetamol-induced liver cirrhosis and fibrosis [13]. Pretreatment with TQ blocked the LPS-induced pro-inflammatory response in LX2, an immortalized human hepatic

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stellate cell line, as demonstrated by a reduced IL-6 and MCP-1 mRNA expression [203]. It also blocked the bleomycin-induced pulmonary fibrosis in rats by attenuation of bleomycin-induced oxidative stress and through NF-κB inhibition [60].

These results suggested that TQ can reduce fibrosis and inflammation, and may be a potential candidate for fibrosis therapy. However, the exact effects of TQ on fibrosis in organs including liver, lung, skeletal muscle, heart, and kidney are not yet clear; and efforts in this direction are still needed.

1.17. Molecular mechanisms

The TQ mechanisms of action are complex because various actions have been observed. TQ has been shown to target multiple factors in many pathophysiological conditions. These pathways include cell cycle progression, proliferation, apoptosis, angiogenesis, migration, invasion and metastasis of tumor in cancer models. Moreover, it inhibits oxidative damage of cellular components and inflammatory responses. TQ also modulates proteins, which involved in metabolism processes. The molecular targets modified by TQ are briefly discussed here below and summarized in Table 3.

1.17.1. Cell cycle and proliferation

Uncontrolled growth and proliferation of cancer cells are important features in carcinogenesis, which causes increase in size of tumor and become problematic to cure. Expression and/or activity of cell cycle progression and proliferation regulators is affected by TQ, leading to cell cycle detention, DNA damage and apoptosis. Evidence indicates TQ has the ability to arrest cancer cells at different phases of the cell cycle; G0/G1 [93,113,205], G1/S [101] and G2/M [97,113].

1.17.2. Apoptosis

P53 is related to cell cycle regulation and apoptosis induction, and TQ induced both p53-dependent [97,108] and p53-independent [119,205] mechanisms of apoptosis. TQ plays a major role in repression of cancer *via* induction of pro-apoptotic factors and/or down-regulation of anti-apoptotic proteins. It moreover regulates the caspase pathways [79,108,112,120,205].

Apoptosis may occur also as a consequence of ROS production. TQ is an anti-oxidant or pro-oxidant depending on its concentration. TQ is anti-oxidant at lower concentrations and most of the studies elucidating the mechanism have addressed on the anti-oxidant effects. However, TQ has been shown at least in one report to be pro-oxidant at high concentrations. TQ is known to induce apoptotic cell death *via*, in part, direct involvement of oxidants [206].

The anti-oxidant/pro-oxidant role of TQ depends also on the environment where it is present [109]. TQ with its respective semiquinone radicals may induce apoptosis in cancer cells by generating ROS [123]. TQ generates ROS and causes low expression of pro-survival genes, conformational changes in pro-apoptotic proteins, loss of mitochondrial membrane potential leading to activation of caspase-9, -3, and PARP cleavage and caspase-dependent apoptosis [106,111,120]. These results place TQ in a class of plant-derived anti-oxidants, which also exhibit pro-oxidant properties.

1.17.3. Angiogenesis

Of relevance to tumor growth, angiogenesis is essential for supplying oxygen and nutrients. A major finding of TQ is that it has potent anti-angiogenic effect. TQ is a selective blocker of VEGF, a key pro-angiogenic molecule, and abolished proliferation and tubulogenesis of endothelial colony forming cells [207]. Endothelial cell migration shows a critical step in the angiogenesis, and TQ effectively inhibited human umbilical vein endothelial cell (HUVEC) migration, invasion, and tube formation [100]. It blocked angiogenesis *in vitro* and *in vivo*, so preventing tumor growth [102,118].

1.17.4. Migration, invasion and metastasis

The most lethal feature of cancer is its ability to spread or metastasize to distant sites. TQ was reported to control invasion and metastasis of cancer [105,116,118,121].

1.17.5. Inflammation and Oxidative stress

The alleviation of oxidative stress and inflammation is a rational strategy to prevent various chronic diseases. Cells are equipped with a wide array of cytoprotective factors, which upon activation protect cells from oxidative and inflammatory insults. TQ have been shown to induce the expression and/or activities of cytoprotective proteins involved in cellular anti-oxidant defense and/or the inactivation of electrophilic carcinogens, thereby preventing oxidative stress [145,146,164,165]. It also acted on the immune system by modulating the levels of immune mediators [68,70].

TQ down-regulates the expression of pro-inflammatory mediators such as COX-2 [73,196], iNOS [84,196], 5-lipoxygenase [31,67,70], TNF-α [22,72] and inhibit the activation of transcription factor NF-κB [77,118], Akt and ERK signaling pathways [100].

Overall, it is likely that TQ modifies a number of molecular targets; however, the molecular mechanisms underlying the effects of the drug remain not fully understood and further work is needed also in this respect.

1.18. Safety and adverse effects

The growing interest in phytomedicine; brings along the issue of their safety, and the legal requirements to meet health standards. It has been shown that the seeds and oil of NS plant are characterized by a very low degree of toxicity [1]. Many studies were carried out to assess the toxicological properties of TQ *in vitro* and *in vivo* [37,151,203], and only a limited number of reports on the potentially toxic effects of TQ exist.

In the pathological conditions where TQ has been shown to be a promising prophylactic it also has been shown to be endowed with a relatively low toxicity [26,29,30,145].

TQ is a very well-tolerated drug in mice. Gali-Muhtasib et al. [107] showed that TQ administered for 20 consecutive days did not induce death in Balb/c mice or affect their mean body weight, which is a very sensitive parameter for toxicity in rodents. TQ was not associated with any clinically important changes in neurological function, laboratory variables or vital signs. TQ administered at 1 mg/kg/day was mostly well tolerated [180]. Badary et al. [208] indicated that addition of TQ in the drinking water of mice at concentrations of up to 0.03% for 3 months led to no sign of toxicity, except for a decrease in fasting plasma glucose concentration. Sustained delivery of TQ for 30 days using Tri-Calcium Phosphate Lysine (TCPL) capsule loaded with 0.02 grams of TQ to adult male rats have shown little or no side effects on the major vital and reproductive organs [190].

It is notable that the effective dose of TQ was found to be safe and no toxicity was reported in subchronic administration of TQ in rats with doses of 90 mg/kg/day [208]. The same authors reported hypoactivity and difficulty in respiration as signs of toxicity at high doses, 24 h after TQ administration at 2–3 g/kg, with a decrease in tissue (liver, kidneys, and heart) GSH content, causing liver and kidney toxicity. This toxicity evidenced by rise in plasma metabolites and enzymes; plasma urea and creatinine concentrations, and the enzyme activities of ALT, LDH, and creatine phosphokinase were increased [37]. Moreover, it has shown TQ increased the rate of necrotic cells at concentrations between 2.5 and 20 μM. Furthermore, it caused concentration dependent genotoxic effect in

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Table 3
Molecular mechanisms underlying activities of TQ (↓: reduction; ↑: increase).

Processes	Molecular targets/pathways	Experimental models/Cell types	Refs
Cell cycle and cell proliferation	↓ PCNA, Ki67, cyclin D1, cyclin E, Cdk4; ↑ p21 → G1/S transition arrest	NDEA-induced hepatocellular carcinoma	[150]
	↑ p53 and p21; ↓ cyclin D1 → induction of G1 phase cell cycle arrest	HCT116 colorectal cancer cells	[108]
	↑ p21 and p27; ↓ E2F-1 and androgen receptor; ↓ Cdk-4, Cdk-2 and cyclin A → blockade of G1/S phase	LNCaP prostate cancer cells	[101]
	↑ Expression of the CDK-inhibitor p16; and ↓ cyclin D1 expression → G0/G1 phase arrest	Papilloma (SP-1) cells	[113]
	↓ IL-6-induced Akt phosphorylation and ↓ IL-6-induced STAT3 expression; ↓ c-Src and JAK-2 activation; caspase-3 activation and PARP cleavage → sub-G1 accumulation	U266 multiple myeloma cells	[79]
	↑ PTEN; ↓ PI3K/Akt pathway; ↑ p53 and p21 protein expression; ↓ cyclin B1 and cdc25 levels → G2/M cell cycle arrest	MCF-7/DOX breast cancer cells	[97]
Apoptosis	↑ PPAR-γ and PPAR-β/δ; activation of caspase-7, -8 and -9; ↓ expression of Bcl-2, Bcl-xl and surviving	MCF-7 breast cancer cells	[95]
	↓ c-myc expression; ↓ β-catenin translocation; ↓ phosphorylation of Akt and GSK3β; ↓ MEK1/2 pathway	Apc^{Min} mice colorectal cancer	[110]
	↓ CHEK1 (checkpoint kinase 1 homolog); ↑ caspase-3 activity → DNA damage and apoptosis	$p53^{-/-}$ HCT116 colorectal carcinoma cells	[108]
	↑ Bax and caspase-3; ↓ Bcl-2 and Bcl-xL; ↓ Mcl-1, survivin and XIAP	HPAC human pancreatic cells	[112]
	↑ Bax/Bcl2 ratio, ↑ release of cytochrome c from mitochondria; activation of caspase-3 and -9; ↓ expression of XIAP	Neuro-2a neuroblastoma cell line	[106]
	↓ Cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1	U266 multiple myeloma cells	[101]
	↓ bcl-2; ↑ bax; activation of caspase-3 and -9; ↑ release of cytochrome c from the mitochondria	Xenograft mouse model of gastric cancer	[111]
	↓ Expression of Bcl-2, Bcl-xL, survivin and Mcl-1 proteins, PARP cleavage	U266 multiple myeloma	[79]
	↑ Levels of p53 and Bax	U87 MG and T98G malignant glioblastoma cells	[104]
	↑ Expression of cytochrome c; ↑ Bax/Bcl-2 ratio; ↓ phosphorylation of Akt and JNK	A431 and Hep2 squamous cell carcinoma cell lines	[114]
	↑ Activation of caspases and PARP cleavage; ↑ Bax/Bcl2 ratio	MCF-7/DOX cells	[97]
	↑ Bax and cytoplasmic cytochrome c; activation of caspase-3; ↓ Bcl-2 and survivin; ↓ phosphorylation of Akt and GSK3β; ↓ EGF-induced phosphorylation of Akt, PTEN, PDK1, and Bad	T47D and MDA-MB-468 breast cancer cells	[96]
	↓ Phosphorylation of JNK and ERK; ↑ activation of caspase-3, and -7	DLD-1 human colon cancer cells	[109]
	↓ IκBα degradation and phosphorylation; ↓ p65 phosphorylation and nuclear translocation; ↓ TNF-α-induced NF-κB-regulated gene products, including IAP1, IAP2, XIAP, Bcl-2, Bcl-xL, survivin, COX-2, cyclin D1, c-Myc, MMP-9 → cell death and ↓ tumor growth	Human chronic myeloid leukemia KBM5 cells	[118]
	↓ Constitutive activation of AKT; activation of caspase-9, -3; and sensitized TRAIL-mediated apoptosis	Primary effusion lymphoma cells	[120]
	↓ Bcl-2, Bcl-xL and survivin; ↑ PPAR-γ	MDA-MB-231 breast cancer cells	[95]
	↑ α and β tubulin degradation; ↑ p73 expression → cell apoptosis	Human astrocytoma cells and in Jurkat cells	[119]
	↓ NF-κB, Ki67, XIAP and survivin; ↑ expression of cleaved caspase-3 and Smac; ↓ expression of CD34 and VEGF	Osteosarcoma (SaOS-2) cells xenograft tumors in nude mice	[102]
	↓ Telomerase activity; induction of DNA damage	M059J and M059K human glioblastoma cells	[103]
Angiogenesis	↓ VEGF expression and ↓ VEGF-induced AKT/ERK activation	Xenograft human prostate cancer (PC3) model in mouse	[100]
	↓ TNF-α-induced expression of VEGF	Human chronic myeloid leukemia KBM5 cells	[118]
	↓ NF-κB pathway, ↓ AKT and ERK signaling pathways, ↓ constitutive and IL-6-induced STAT3 phosphorylation	U266 multiple myeloma cells	[79]
Cell migration, invasion and metastasis	↓ Chemokine receptor-4 (CXCR4), COX-2 MMP-9 and p65 expression	U266 multiple myeloma cells	[121]
	↓ Expression of inflammosome marker NLRP3; ↓ secretion of IL-1β and IL-18; ↓ NF-κB activity	B16F10 melanoma cells in C57/BL6 mice	[116]
	↓ MMP-2 and -9; ↓ ERK phosphorylation; ↓ FAK	U87 and CCF-STTG1 glioblastoma cells	[105]
	↓ TNF-α-induced expression of MMP-9	KBM5 myeloid leukemia cells	[118]
Inflammatory responses	↓ IL-4, -5 and -13; ↓ LTB4 and LTC4 levels; ↑ IFN-γ	Mouse model of allergic airway inflammation	[69]
	↓ IL-1β, IL-6, TNF-α, IFN-γ and PGE2; ↑ IL-10	Collagen-induced arthritic rats	[72]
	↓ TGF-β1, iNOS, COX-2 and prostaglandin expression	Allergic airway inflammation	[68,70]
	↓ TNF-α and IL-2 levels	LPS and live *E. coli*-induced sepsis	[135]
	↓ NO, MDA, IL-1β, and TNF-α levels	Spinal cord injury (SCI)	[178]
	↓ IL-6 and NF-κB activation	Encephalomyelitis model	[73,76]
	↓ COX-2 expression; ↓ PGE2 accumulation and ↓ activation of NF-κB	HPAC human pancreatic cancer cells	[112]
	↓ COX-2; ↓ NF-κB signaling (phosphorylation of Akt, JNK and p38 MAP kinase)	HR-1 hairless mouse skin	[115]
	↓ lipid peroxidation; ↓ expression of COX-2 and MDA; ↑ SOD level	Pancreatic tissue of STZ-diabetic rats	[154]
	↓ LPS-induced IL-1β, TNF-α, MMP-13, COX-2; ↓ PGE2, NF-κB, p65, p38 and ERK1/2 phosphorylation	Rheumatoid arthritis	[75]
	↓ PKC, PAF, histamine release and iNOS expression; ↓ GSH depletion and lipid peroxidation	Peritoneal mast cells	[80]
	↓ expression of PI3K, CD14 and TLR4, collagen-I and Akt phosphorylation	Activated rat hepatic stellate cell line, T-HSC/Cl-6	[203]
	↓ expression of brain iNOS and NO	Morphine-induced oxidative stress	[171]
	↓ NO and iNOS expression and/or production	Supernatants of LPS-stimulated macrophages	[84]
	↓ MDA and NO level; ↑ activities of CAT, GSH-Px and SOD	Acute bacterial prostatitis induced by *P. aeruginosa* or *E. coli*	[136,137]
Oxidative stress			

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Table 3 (*Continued*)

Processes	Molecular targets/pathways	Experimental models/Cell types	Refs
	↓ Level of MDA; ↑ GSH content, CAT and SOD activities; ↓ lipid peroxidation	I/R injury in rat hippocampus	[34]
	↑ GST, GSH-Px, SOD and CAT	Hypercholesterolemia	[27,50]
	↓ NO and MPO; ↑ GSH, CAT and SOD	Collagen-induced arthritis	[72]
	↑ HO-1 and GST	HR-1 hairless mouse skin	[115]
	↑ Content of GSH and SOD activity; ↓ MDA content and MPO activity; ↓ NO production	I/R-induced gastric dysfunction and ulcer in rats	[164,165]
	↓ NO and MDA; ↑ GSH and CAT; also ↓ norepinephrine and dopamine	STZ-diabetes model	[162]
	↓ lipid peroxidation; ↓ AST and ALT activity	Cypermethrin-induced hepatotoxicity	[147]
	↓ AST, ALT, ALP and MDA levels	Aflatoxin B1-induced hepatotoxicity	[57]
	↑ GSH content, and GST and DT-diaphorase activities; ↓ lipid peroxide accumulation	Liver of B(a)P-treated tumor-bearing mice	[61]
	↑ SOD, CAT, GSH and non-protein thiol (NP-SH) levels	Cadmium-induced hepatotoxicity	[145]
	↓ AST, ALT and ALP levels	Anti-tuberculosis drugs-induced liver damage	[148]
	↓ MDA; ↑CAT, SOD and GST-Px	1,2-Dimethyl-hydrazine (DMH)-induced colon tumor	[63]
	↓ MDA, ↑ activities of SOD and GST; along with ↓ NOX-4 level	DOX-induced nephrotoxicity	[187]
	↓ TBARS; ↑ GSH level, GSH-Px and CAT activities	Gentamicin-induced acute renal failure in rats	[52]
	↓ Hepatic GSH depletion; ↑ activity of SOD, ↓ TNF-α	Tamoxifen-induced hepatotoxicity in female rats	[146]
	↓ Kidney tissue MDA; ↑ activities of SOD and GSH-Px in kidney tissue	Vancomycin-induced kidney injuries	[53]
	↓ ALT activity; ↑ GST as well ATP production	Acetaminophen-induced hepatotoxicity	[56]
	↑ Hepatic LDL receptor gene; ↓ 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR) gene → hypocholesterolemic effect	Diabetic rats	[158]
Components involved in metabolism	↓ Serum lipase, amylase, MPO, and oxidative stress index	Ethanol and high fat diet	[201]
	↓ Total cholesterol, LDL-cholesterol, triglycerides and TBARS; ↑ HDL-cholesterol concentration	Cholesterol-fed rabbits	[199]
	↓ Activities of glucose-6-phosphtse and fructose-1,6-bisphosphatase → ↓ gluconeogenesis	STZ-nicotinamide-induced diabetic rats	[25]
	↑ Alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase	Adjuvant-induced arthritis rat model	[75]
	↑ ALP, osteocalcin, osteopontin and BMP-2; ↑ phosphorylation of ERK signaling pathway and activated MAPK pathway → osteogenesis	MC3T3-E1 osteoblast cells	[192]
	↓ MDA level; ↑ GST and SOD activity in liver and kidney tissues; ↓ spermidine/spermine N-1-acetyl-transferase (SSAT) and ↓ CYP3A1 gene expression in liver and kidney	Hepatorenal dysfunction induced by renal I/R	[188]
	↓ Creatine kinase, LDH, cholesterol, and TNF-α; ↓ TBARS; and ↑ ATP levels	Cyclophosphamide-induced cardiotoxicity	[58]

hepatocyte primary cultures, *i.e.* an increase of the frequency of chromosomal aberrations and micronucleated cells [151].

Importantly however, TQ had selective cytotoxic effects and also these, as shown above, have potential therapeutic application. In this respect, it appears that TQ kills tumor cells efficiently without cytotoxicity to normal cells. The selective cytotoxicity of TQ for malignant cells compared to normal osteoblasts [205], mouse normal kidney cells [93], normal human lung fibroblasts [103] and Vero cells [99] has been described. In addition, normal cell lines such as primary mouse keratinocytes and Madin-Darby canine kidney (MDCK) cells are reported to be resistant to the cytotoxic effects of TQ (IC_{50} = 101 μM) [16,113].

TQ, while depolymerized the microtubule network and disrupting the mitotic spindle organization of A549 cells, did not affect the microtubule network of the more normal HUVEC cells and at below the IC_{50} concentration [122]. TQ degraded α and β tubulin of human astrocytoma and Jurkat cells, without affecting normal human fibroblast cells [119].

Not all is, however, straight forwards, as mentioned above TQ might be metabolized to reactive species and increase oxidative stress, which contributes to the depletion of anti-oxidant enzymes and damage to DNA in hepatocytes treated with high TQ concentrations [151]. TQ triggered suicidal erythrocyte death, an effect paralleling the apoptotic effect on nucleated cells. Exposure of human erythrocytes to TQ stimulated phosphatidylserine exposure at the erythrocyte surface. The effects are expected to accelerate the clearance of erythrocytes from circulating blood, thus predisposing to the development of anemia [203].

The route of administration could have an influence on TQ toxicity outcome. Rats that received intraperitoneal TQ showed toxicity signs, which were related to acute pancreatitis. Meanwhile, rats, which received oral ingestion, showed transient toxicity. This can be explained by the fact that intraperitoneal injection resulted in whole absorption of TQ into systemic circulation; whereas with oral ingestion, TQ was biotransformed in gastrointestinal tract or metabolized in the liver [37].

The lack of preclinical studies with TQ reporting the maximum tolerated dose (MTD), which is defined as the highest dose is safe to administer in the absence of intolerable side effects, is regarded as a limitation in using TQ in clinical settings [37]. Further research both at the clinical and preclinical level is thus needed to determine the therapeutic effective dose of TQ in various diseases. In particular, the effect of TQ on ionic channels, especially calcium channels, is still unclear and needs to be more investigated.

2. Discussion and future viewpoints

Nowadays extensive research is focusing on herbal products as an alternative medicine and traditional medicinal plants have received much attention due to several factors such as cheap cost, easy availability, safety, and efficacy. Moreover, many plants and their products are used based on religious and cultural traditions. *N. sativa* seeds, from which TQ is extracted, have been used by diverse human cultures around the world for centuries to treat many problems [4].

TQ, as an example of phytochemicals, has attracted noteworthy scientific attention in recent years for its high biological activity and low systemic toxicity that can make it a promising alternative to conventional therapeutic drugs. Plant-based anti-oxidants have recently gained popularity due to their role as dietary supplements with minimal side effects. Additionally, the use of naturally occurring agents to prevent the development or recurrence of cancers has become widely accepted as a realistic option for fighting diseases. The need for new treatments for cancer, which have fewer

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adverse effects, has generated interest in exploiting the anti-cancer properties of dietary phytochemicals and other natural products. A potential anti-cancer drug should exhibit selective and specific cytotoxicity against cancerous cell leaving the normal cells unharmed. Extensive results show that TQ has these properties.

There is no doubt that *in vitro* and *in vivo* research has demonstrated the therapeutic potential of TQ against cell cultures and animal models with the emphasis on the mechanism of action. However, there are no clinical data upon which to base dosing recommendations in humans and current understanding about its action in the body is not satisfactory. There is a paucity of clinical studies testing TQ in human patients; We believe that it is now appropriate that TQ should move from testing on the bench to clinical experimentation, in which explore effects of TQ on immune system, liver as well as upon other vital tissues, areas in which its potential toxicity has emerged.

Nevertheless, considerable amount of information about TQ regarding its molecular activity, drug toxicity, and novel drug delivery approaches are now available for researchers. Results emerging from such studies will substantially improve the therapeutic application of TQ in clinical settings for a wide range of illnesses once we have clear information on the conditions for its safe use. The efficacy of TQ should be measured based on the nature of the diseases. As detailed above TQ may synergize with more conventional chemotherapeutic drugs. Its use in combination therapies may be at lower dosage, reduce the dosage of the concomitant drug optimizing efficacy *vs* toxicity; it might also overcome to drug resistance problem [112,123].

Conflict of interest statement

None declared.

Uncited reference

[204].

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