Thymoquinone in Chemotherapy

Subjects: Biochemical Research Methods

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Thymoquinone (2-Isopropl-5-methyl benzo-1,4-quinone) is an active component of the volatile oil of Nigella sativa (black seeds). Other than the Ranunculaceae family (N. sativa), this compound has been detected in other families such as Lamiaceae (Modernadidyma, M. menthifolia, etc.), Asteraceae (Eupatorium cannabinum), and Cupressaceae (Juniperus communis).

Keywords: Thymoquinone ; chemotherapy-induced toxicity ; antioxidant

1. Thymoquinone (TQ)

Thymoquinone has a broad range of pharmacological actions involving anti-oxidative, anti-inflammatory, immunomodulatory, anti-cancer, anti-microbial, hepatoprotective, hypoglycemic and antidiabetic, gastroprotective, neuroprotective, cardioprotective, nephroprotective, hypolipidemic, and anti-histaminic effects. It also showed a protective effect on reproductive system, respiratory system, and bone related disorders [1]. TQ is generally safe to use but toxicity has been observed at higher doses (LD_{50}, 2.5 g/kg). In a study conducted by Abukhader, Wistar Albino rats administered with 30 mg/kg and 40 mg/kg of TQ showed signs of toxicity such as lethargy, abdominal swelling, piloerection, irritability, and weight loss. It was found that 22.5 mg/kg and 15 mg/kg are the maximum tolerated doses (MTD) through the intraperitoneal route (I.P) in male rats and female rats, respectively. When administered orally at doses of 500 mg/kg and 300 mg/kg, toxicity signs like dyspnea, abdominal distension, weight loss, diarrhea, and hypoactivity were observed. The MTD through oral route (P.O) for both male as well as female rats were reported as 250 mg/kg. MTD is the highest safe dose that can be administered without showing signs of toxicity [2].

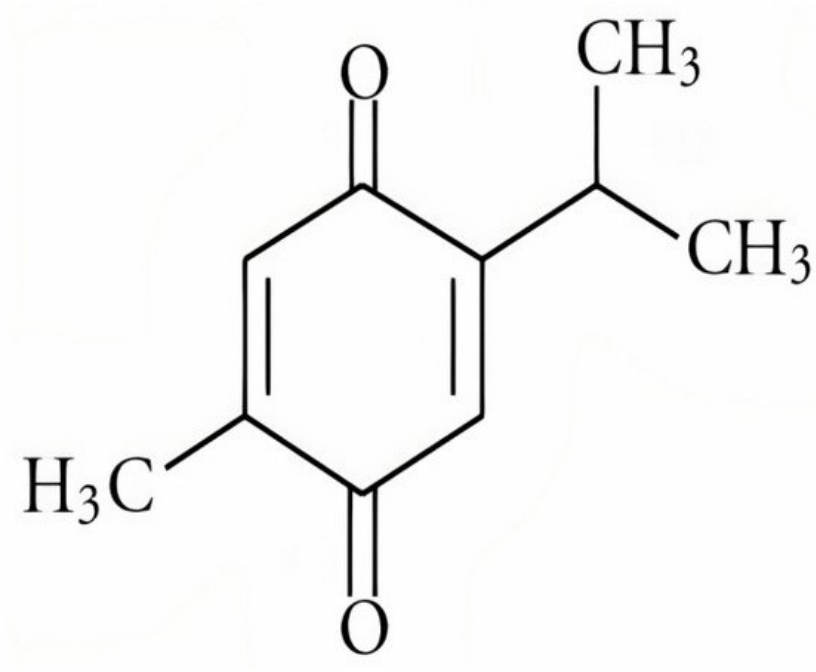

Figure 1. The chemical structure of thymoquinone [3].

Figure 2. *Nigella sativa* (**a**)flower (**b**) seeds [4][5].

1.1. Role of Thymoquinone in Cancer

There are many preclinical studies (both in vivo and in vitro) which demonstrate the effect of TQ, given alone or in combination with other chemotherapeutic agents. It is known to target various processes of the cancer model such as cell cycle progression, proliferation, apoptosis, angiogenesis, migration, invasion, and metastasis of tumors. It also prevents oxidative damage and inhibits inflammatory responses [1]. TQ showed therapeutic efficacy against a wide range of cancer including ovarian cancer [6][7], breast cancer [8][9], pancreatic cancer [10], lung cancer [11][12], fibrosarcoma [13], neuroblastoma [14], osteosarcoma [15], myeloma [16], oral cancer [17], colon cancer [18][19], prostate cancer [20], squamous cell carcinoma [21], gastric cancer [22], leukemia [23], cervical cancer [24], liver cancer [25][26], and skin cancer [27]. In addition to its chemotherapeutic effects, it also diminishes the toxic side effects caused by chemotherapeutic agents.

1.2. Effect of TQ against ChemotherapyInduced Organ Toxicity

Toxicity is a major concern for chemotherapeutic drugs. Some of the most widely used chemotherapeutic drugs possess organ toxicity which leads to further economic burden and decreased quality of life. Isolated phytoconstituents are equipotent to synthetic analogues and have lesser side effects.

1.2.1. Protective Effect against Cardiotoxicity

Many studies have demonstrated that TQ has a cardioprotective effect against chemotherapeutic drug-induced cardiotoxicity. Doxorubicin is an antitumor antibiotic used in the treatment of various types of cancer but its use is restricted due to the dose limiting cardiotoxicity. Alam et al. [28] conducted a study which demonstrated that serum enzyme markers of cardiotoxicity such as AST, ALT, CK-MB, and LDH in doxorubicin treated groups were elevated. MDA, a final product of lipid peroxidation, which act as an indicator for oxidative stress was increased and antioxidant enzymes such as CAT, SOD, GPx, GR, GST and GSH were decreased in doxorubicin treated groups. Oxidative stress occurs due to increase in reactive oxygen species generation and/or weakened antioxidant defense mechanism. Upon TQ treatment in mice (10 mg/kg, 20 mg/kg p.o), elevated levels were restored to normal. They also suggested that restoration may be due to the high antioxidant property of thymoquinone in the myocardium, thus minimizing the damage to the cardiac muscle fibers caused by doxorubicin. Co-treatment with TQ also restored the elevated levels of inflammatory cytokine (IL2) in cardiac tissue. These results were in accordance with other studies on doxorubicin-induced cardiotoxicity [29][30][31]. Karabulut et al. [31] performed a histopathological examination which revealed myocardial fibril disorganization in DOX-treated groups which was almost normally organized after treatment with TQ (10 mg/kgi.p). ANP and NT-proBNP values were determined, revealing that a doxorubicin dose of 15 mg/kg, i.p resulted in cardiac dysfunction leading to heart failure. These values were decreased by administration of thymoquinone before and after DOX-treatment. TQ supplementation also prevented cyclophosphamide-induced cardiotoxicity by restoring the abnormal levels of serum cardiac markers, lipid peroxidation, antioxidant enzymes and inflammatory mediators [32]. Cisplatin treatment caused myocardial tissue necrosis and apoptosis as evidenced by histopathological examination, which was diminished after TQ administration [33].

1.2.2. Protective Effect against Hepatotoxicity

Tamoxifen is commonly recommended for treatment of patients with breast cancer [34]. It has the unusual side effect of producing hepatotoxicity, making long-term use challenging. Treatment with tamoxifen to rats resulted in significant increase in ALT, ALP, AST, LDH, γGT and total bilirubin. Histopathological changes included swelling of interstitial tissues and inflammation of Von Kuppfer cells with atrophy. There was a significant increase in lipid peroxidation level, inflammatory marker (TNF-α) and a significant decrease in antioxidant levels. All these changes ameliorated after administration of TQ [35]. It also showed hepatoprotective activity against cisplatin, cyclophosphamide and methotrexate

toxicity with similar improvements in hepatic serum markers, lipid peroxidation, inflammatory markers, and antioxidant levels [36][37][38]. As a defence mechanism, inducible nitric oxide synthase (iNOs) is known to produce significant levels of nitric oxide; however, the excess production may cause damage to the liver. El-Sheikh et al. [38] performed immunohistochemical staining of the liver in methotrexate-treated rats and it was observed that iNOs expression upregulated, which confirmed nitrosative stress. Nitric oxide levels were also increased in cisplatin-induced hepatotoxicity [36]. These effects were reversed after treatment with TQ, suggesting that it has an antinitrosative effect. All these data show that TQ has hepatoprotective activity and can be used as an adjuvant in treating hepatotoxicity caused by chemotherapeutic drugs.

1.2.3. Protective Effect against Nephrotoxicity

Literature survey revealed the protective effect of thymoquinone against nephrotoxicity in rats when given in combination with doxorubicin, cisplatin and ifosfamide. Badary et al. [37] demonstrated that doxorubicin treatment produced renal changes as manifested by hypoproteinemia, proteinuria, albuminuria, hyperlipidemia, hypoalbuminemia. They also evaluated oxidative stress as examined by lipid peroxide generation, non-protein sulfhydryl (NPSH) concentration and catalase (CAT) activity in renal tissue. These changes were improved after TQ administration to nephritic rats. A study was conducted on both rats as well as mice by Badary et al. [38] revealing that the rats were more sensitive to cisplatin-induced nephrotoxicity than mice. It was found that serum urea and serum creatinine levels were elevated with decreased creatinine clearance, increased urinary volume and renal tubular damage was also observed in the cisplatin treated group. Similar changes were also observed in ifosfamide-induced nephrotoxicity [39]. Upon TQ administration, the abnormal levels were restored to normal. TQ showed protection against doxorubicin-induced nephrotoxicity by improving lipid peroxidation, antioxidant levels, nitrosative stress markers as well as inflammatory markers [40].

1.2.4. Protective Effect against Intestinal Toxicity

Methotrexate is known to cause serious intestinal toxicity. A study has demonstrated the mechanism by which TQ shows protection against methotrexate-induced intestinal toxicity. It acts by decreasing oxidative and nitrosative stress markers in intestinal mucosa, decreasing expression of inflammatory markers (TNF-α, NF-κB and COX-2) and by inhibiting apoptosis [41]. Another study by Shahid et al. [42] estimated the effect of long-term cisplatin use in rat intestines and the role of TQ in preventing toxicity. It was observed that repeated doses of cisplatin increased MDA levels, decreased GSH and TSH levels, as well as other antioxidant enzymes such as SOD, GSH-Px, CAT, GST, GR and TR in intestinal mucosa. Glutathione peroxidase, glutathione reductase, and glutathione-s-transferase are the main GSH-dependent antioxidant enzymes. Histopathology of brush border mucosa (BBM) revealed morphological changes such as congestion, swelling of villi, alteration in the contour and increased lymphocytic infiltration in the lamina propria associated with reduction in the crypt/villus ratio. It was further noticed that there was a significant reduction in enzyme markers of brush border mucosa which are sucrase, ALP, GGTase, and LAP. They also observed alterations in enzymes involved in carbohydrate metabolism. These abnormalities in both methotrexate and cisplatin were restored to normal upon TQ administration.

1.2.5. Protective Effect against Urotoxicity

Cyclophosphamide-induced hemorrhagic cystitis is the dose limiting toxicity causing damage to urothelium of bladder mucosa. However, administration of TQ has shown protective activity against it by increasing antioxidant enzymes, decreasing lipid peroxidation, reducing levels of inflammatory markers, and maintaining the structure and morphology of the urinary bladder by increasing Nrf2 expression. Oxidative stress causes the level of Nrf2 to decrease [43].

1.2.6. Protective Effect against Ototoxicity

Sagit et al. [42] demonstrated that cisplatin-induced ototoxicity in rats is caused due to an increase in ABR thresholds and a decrease in DPOAE responses. The intensity of sound at which a brain response initially appears is determined by ABR analysis [44]. DPOAEs are emission of sounds in response to two tons of different frequencies played at the same time [45]. Accumulation of cisplatin in cochlear tissues generates excessive free radicals and decreases antioxidant enzymes which results in cochlear injury or cell death. TQ administration preserved the changes produced by cisplatin treatment [46].

1.2.7. Protective Effect against Testicular Injury

Gokce et al. [47] identified rise in TAC level and myeloperoxidase activity in methotrexate treated groups. An increase in TAC is a result of compensation to oxidative stress generated while increased myeloperoxidase activity indicates neutrophil accumulation leading to oxidative testicular damage. The seminiferous epithelium was severely disrupted, reducing the diameter of the seminiferous tubules and affecting the spermatogenetic cell lines. It also caused interstitial space dilation and edema in mice with cell size reduction and cytoplasmic swelling. TQ exerted its protective effect by restoring the abnormalities caused produced by methotrexate treatment.

1.2.8. Protective Effect against Pulmonary Toxicity

A study was conducted by Suddek et al. [48] to demonstrate acute pulmonary toxicity induced by cyclophosphamide. They observed increase in MDA levels, SOD (as a compensatory mechanism), total protein, serum LDH, TNF-α and a decrease in GSH. Histopathological examination of the lung revealed congestion, damage, swelling of interalveolar septum, neutrophilic, and macrophages infiltration. TQ supplementation resulted in reversal of the changes produced by Cyclophosphamide.

2. Role of Thymoquinone against Chemotherapy Induced Oxidative Stress

Reactive oxygen species (ROS) are produced as a result of various cellular processes involving endogenous and exogenous pathways, constituting oxidative stress [49]. Endogenous pathways include oxidative phosphorylation, bacterial invasion, and inflammation; whereas exogenous pathway includes xenobiotics, radiation, and pollution. ROS are implicated in the anticancer mechanism of chemotherapy which further causes adverse effects. In other words, it has been established that chemotherapeutic agents have the potential to induce oxidative stress during the treatment [50]. Conklin [51] reported that among anticancer drugs, oxidative stress was observed to be the highest in theanthracycline class of drugs followed by platinum compounds, alkylating agents, epipodophyllotoxins and camptothecins but was lower in taxanes, antimetabolites, and vinca alkaloids. Hence the generation of ROS following the administration of chemotherapeutic agents decreases their efficacy. Inorder to prevent the side effects and to enhance responsiveness to therapy, antioxidants are recommended. SOD and CAT are antioxidant enzymes which are primarily responsible for destroying reactive oxygen metabolites. Glutathione enzymes (GSH, GPx, GR, GST) have an important role in providing secondary defense against oxidative damage caused by the generation of ROS [52]. Previous studies described how the supplementation of thymoquinone to experimental animals treated with chemotherapeutic agents increased antioxidant enzyme activity and thus showed some protection against organ damage.

3. New Trends and Directions of Research Related to TQ

Due to poor pharmacokinetic characteristics of TQ, its use in humans is limited as it is rapidly eliminated and slowly absorbed. To enhance bioavailability, several researchers developed nanoformulations of TQ which showed marked improvement in pharmacokinetic properties. In 2017, the United States government registered a clinical trial which evaluated the chemopreventive effect of TQ on oral potentially malignant lesions. Currently there are three completed trials and two ongoing trials registered for thymoquinone [53].However, there are no registered clinical trials on role of thymoquinone in reducing the toxicity of anticancer drugs. There are sufficient in vitro and in vivo studies describing the potential of TQ in reducing chemotherapeutic drug-induced toxicity, hence undertaking future clinical investigation in this particular area would be interesting in order to improve the efficacy of chemotherapy by diminishing the adverse effects produced during treatment.

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