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Thymoquinone Supplementation Induces Quinone Reductase and Glutathione Transferase in Mice Liver: Possible Role in Protection against Chemical Carcinogenesis and Toxicity

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Thymoquinone (TQ), the main constituents of the volatile oil from Nigella sativa seeds is reported to protect laboratory animals against chemical carcinogenesis and toxicity through mechanism(s) that is not fully understood. Among possible mechanism(s), protection could be mediated via induction of detoxifying enzymes, including quinone reductase and glutathione transferase. This study was undertaken to investigate whether oral administration of TQ increases the activities of quinone reductase and glutathione transferase in mice liver. Overdose of TQ, when administered intraperitoneally, caused a marked depletion of hepatic glutathione in both a time- and dose- dependent manner, a characteristic of a group of compounds known as Michael reaction acceptors which are known to act as inducers of enzymes that protect against chemical carcinogenesis and toxicity. TQ was given (1, 2 and 4 mg/kg/day p.o.) for five days to test the chemical inducibility of quinone reductase and glutathione transferase in mice liver. TQ administration produced significant increase in the activities of quinone reductase (147, 196 and 197% of control, respectively) and glutathione transferase (125, 152 and 154% of control, respectively). In conclusion, oral administration of TQ is effective in increasing the activities of quinone reductase and glutathione transferase and makes TQ a promising prophylactic agent against chemical carcinogenesis and toxicity. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: thymoquinone; glutathione and glutathione transferase; quinone reductase.

INTRODUCTION

Thymoquinone (TQ), the main constituents of the volatile oil from *Nigella sativa* seeds is reported to protect laboratory animals against chemical toxicity and induction of carcinogenesis. Previous studies showed that pretreatment with TQ protected organs against oxidative damage induced by a variety of free radical generating agents including carbon tetrachloride evoked hepatotoxicity (Nagi *et al.*, 1999), doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000) and nephropathy produced by cisplatin (Badary *et al.*, 1997). The antitumor effects of TQ have also been reported in different tumor models. Administration of TQ in drinking water resulted in significant suppression of forestomach tumor induced by benzo(α)pyrene (Badary *et al.*, 1999). Similarly, TQ significantly inhibited the tumor incidence of 2-methyclonathrene induced fibrosarcoma (Badary and El-Din, 2001). Furthermore, oral administration of TQ to mice bearing Ehrlich ascites carcinoma xenograft significantly enhanced the antitumor effect of ifosfamide (Badary, 1999).

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Quinones are a ubiquitous class of compounds which are common constituents in plants (Bolton *et al.*, 2000). Quinones react with glutathione via the 1,4-Michael addition mechanism, producing glutathione conjugates *in vitro* (O'Brien 1991, Wang *et al.*, 2006 and Chan *et al.*, 2008). The potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis and chemical toxicity depends on their reactivity with sulfhydryl groups (Dinkova-Kostova *et al.*, 2001). TQ (a quinone compound) was found to react *in vitro* with glutathione through spontaneous and rapid reaction that produces a dihydrothymoquinone-thioether (Khalife and Lupidi, 2007). Therefore, it is reasonable to hypothesize that the TQ administration induces enzymes that protect against carcinogenesis and chemical toxicity. This study was undertaken to investigate whether oral administration of TQ induces the activities of quinone reductase and glutathione transferase in mice liver. In addition, the dose-dependence of the two enzyme activities was also examined.

MATERIALS AND METHODS

Chemicals. Thymoquinone, glutathione, Ellman's reagent [(5′-5′-dithiobis-(2-nitrobenzoic acid) DTNB], bovine serum albumin, 2,6-Dichlorophenolindophenol and β, nicotinamide adenine dinucleotide phosphate were

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purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of the highest analytical grades commercially available.

Animals. Male Swiss albino mice weighing 25–30 g were used in all experiments. They were obtained from the Experimental Animal Care Center, King Saud University, Riyadh, Saudi Arabia. They were housed under conventional laboratory conditions in a room temperature maintained at 25 ± 1 °C and a relative humidity range of 40% to 75% with a regular 12-h light – 12-h dark cycle. The mice were fed a standard animal pellet diet and allowed free access to water unless otherwise indicated. Experiments were approved by a local ethical committee of college of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Experimental protocols. In the first set of experiments, mice were randomly allocated into four groups, each consisting of six animals. The control group received saline while the other three groups received TQ at the dose of 500 mg/kg i.p. TQ was dissolved in warm saline (65 °C) and cooled to room temperature before i.p. use. Animals were sacrificed by cervical dislocation at 0.5, 1 and 2 h following the administration of TQ and the livers were quickly isolated, washed with saline, plotted dry on a filter paper and weighed. Homogenization was carried out in ice-cold saline to yield a 10% (w/v) tissue homogenates and glutathione was assessed.

In the second set of experiments, mice were randomly allocated into four groups, each consisting of six animals. The control group received saline while the other three groups received TQ at the doses of 100, 250 and 500 mg/kg i.p. Animals were sacrificed by cervical dislocation at 2 h following the administration of TQ and the livers were quickly isolated, washed with saline, plotted dry on a filter paper and weighed. Homogenization was carried out in ice-cold saline to yield a 10% (w/v) tissue homogenates and glutathione was assessed.

In the third set of experiments, mice were randomly allocated into four groups, each consisting of six animals. The control group received water while the other three groups orally received TQ at the doses of 1, 2 and 4 mg/kg/day for five consecutive days. TQ was dissolved in warm drinking water (65 °C) and cooled to room temperature before oral use. Animals were sacrificed by cervical dislocation and the livers were quickly isolated, washed with saline, plotted dry on a filter paper and weighed. Homogenization was carried out in 0.25 M sucrose to yield 10% (w/v) tissue homogenates. The initial centrifugation was performed at 700 g for 10 min and the resulting supernatant was centrifuged at 8000 g for 10 min. The supernatant was carefully separated from the pellet and centrifuged at 17 000 g for 10 min. Once again the resulting supernatant (post mitochondrial fraction) was carefully removed from the pellet and used for the determination of quinone reductase and glutathione transferase activities.

Determination of glutathione. Liver tissue levels of acid soluble thiols, mainly glutathione, were determined colorimetrically at 412 nm (Ellman, 1959). Homogenates were precipitated with 5% perchloric acid and after centrifugation at 1000 g for 5 min supernatants were used for the estimation of GSH level. The concentration of GSH is expressed as μmol/g liver tissue.

Assay of quinone reductase. Quinone reductase activity was estimated spectrophometrically according to the method of Ernster (1967) using 2,6-dichlorophenolindophenol and NADPH by measuring the decrease in absorbance at 600 nm. The results were expressed as nmol/min/mg protein.

Assay of glutathione transferase. Glutathione transferase activity was assayed as previously described (Habig *et al.*, 1974) using 1-chloro-2, 4-dinitrobenzene and the results were expressed as nmol/min/mg protein.

Assay of protein. Protein contents were determined by the biuret reaction using bovine serum albumin as slandered (Gornall *et al.*, 1949).

Statistical analysis. Differences between obtained values (mean ± SD, $n = 6$) were carried out by one way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test to assess which treatment groups showed a significant difference from the control group. The differences were considered significant when $p < 0.05$.

RESULTS

The intraperitoneal (i.p.) injection of various quantities of the quinone, TQ, resulted in a substantial reduction in GSH content in liver (Fig. 1). For example 2 h after administration of a dose of 250 mg TQ per kilogram body weight, a 43% decrease in the GSH level was observed. The decrease was dose-dependent and a 500 mg TQ per kilogram body weight caused a 71% depletion of GSH. Time course of GSH depletion after administration of 500 mg TQ per kilogram body weight is shown in Fig. 2. Within 30 min following i.p. injection, nearly 33% of the GSH disappeared; by 2 h, only

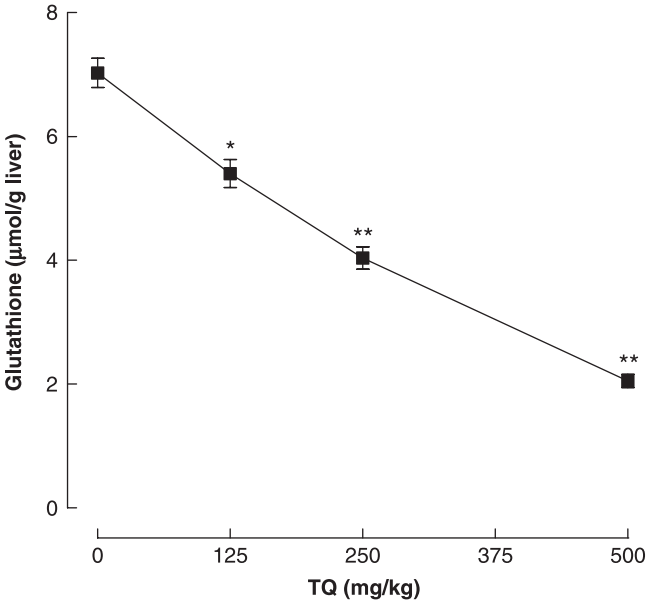

Figure 1. Effect of different doses of thymoquinone on liver glutathione content. TQ was given (125, 250 and 500 mg/kg/i.p.). The hepatic GSH were determined 2 h after varying amounts of TQ. Each bar represents the mean ± SD of six mice.
* $p < 0.05$ compared to dose zero
** $p < 0.001$ compared to dose zero

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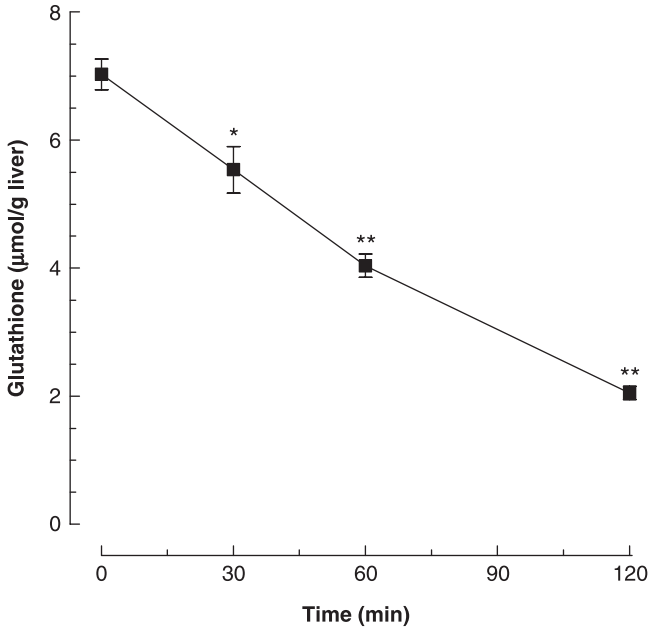

Figure 2. Time course of hepatic glutathione depletion after administration of thymoquinone. TQ was given (500 mg/kg/day i.p.) and mice were sacrificed 0.5, 1.0 and 2 h following the i.p. injection of TQ and the hepatic GSH were determined. Each bar represents the mean ± SD of six mice.
* $p < 0.05$ compared to time zero
** $p < 0.001$ compared to time zero

28% remained. These results suggest that the decline in the level of GSH is due to direct interaction between GSH and the administered TQ confirming the *in vitro* results of Khalife and Lupidi (2007).

Quinone reductase and glutathione S-transferase activities in liver post mitochondrial fraction were measured after TQ administration (1, 2 and 4 mg/kg/day p.o.) for five days. As shown in Fig. 3, TQ administration caused a significant increase in quinone reductase activity (147 197, and 197% of control respectively) and as shown in Fig. 4, TQ administration caused a significant increase in glutathione transferase activity (125 152 and 154% of control respectively).

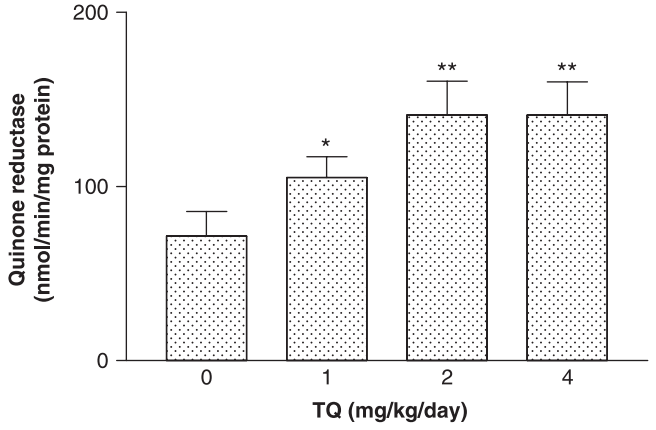

Figure 3. Effect of different doses of thymoquinone on liver quinone reductase activity. TQ was given (1, 2 and 4 mg/kg/day p.o.) for five days. Quinone reductase activity in liver post mitochondrial fraction was measured. Each bar represents the mean ± SD of six mice.
* $p < 0.05$ compared to control
** $p < 0.001$ compared to control

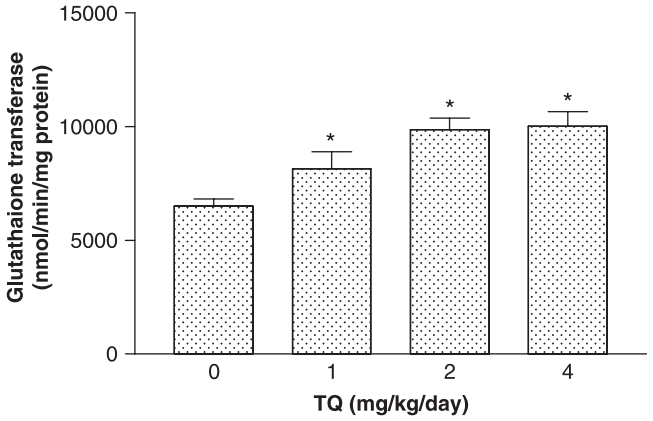

Figure 4. Effect of different doses of thymoquinone on liver glutathione transferase activity. TQ was given (1, 2 and 4 mg/kg/day p.o.) for five days. Glutathione transferase activity in liver post mitochondrial fraction was measured. Each bar represents the mean ± SD of six mice.
* $p < 0.001$ compared to control

DISCUSSION

Overdose of TQ, when administered intraperitoneally, caused a marked depletion of hepatic glutathione in both a time- and dose- dependent manner, a characteristic of a group of compounds known as Michael reaction acceptors which are known to act as inducers of enzymes that protect against chemical carcinogenesis and toxicity (Dinkova-Kostova *et al.*, 2001, Chan *et al.*, 2008). Oral administration of TQ (1, 2 and 4 mg/kg/day p.o. for 5 days) resulted in a significant induction of quinone reductase and glutathione transferase (phase 2 enzymes) activities in mice liver and the inducibility of these enzymes is dose-dependent. These studies suggested that a significant amount of TQ could be achieved in liver tissue in whole animals upon oral administration, leading to increased expression of endogenous phase 2 enzymes. TQ, like all quinones, can be lethal in high doses, due to reactions with cellular nucleophiles, including –SH groups on essential compounds, such as proteins as well as glutathione. This may explain, in part, why TQ is an effective inducer of phase 2 enzymes at lower dose levels (this study) than higher levels (Mansour *et al.*, 2002). Consistent with this conclusion, TQ was used as protecting agent in the range of 1–10 mg/kg/day, orally in the models of chemical toxicity and carcinogenesis (Salem, 2005).

TQ has been shown to protect against oxidative stress in various, highly divergent experimental systems including doxorubicin-induced cardiotoxicity (Nagi and Mansour, 2000); carbon-tetrachloride-evoked hepatotoxicity (Nagi *et al.*, 1999); nephropathy produced by cisplatin (Badary *et al.*, 1997); suppression of forestomach tumor induced by benzo(α)pyrene (Badary *et al.*, 1999); and inhibition of the tumor incidence of 2-methyclonathrene induced fibrosarcoma (Badary and El-Din, 2001). Furthermore, oral administration of TQ to mice bearing Ehrlich ascites carcinoma xenograft significantly enhanced the antitumor effect of ifosfamide (Badary, 1999). Accordingly, induction of phase 2 enzymes by TQ may be an important mechanism underlying the protective effects of TQ observed with these models of chemical toxicity and carcinogenesis. Some studies suggest that

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the enhancement of phase 2 enzymes by antioxidants, such as polyphenols present in plant-water extracts, is achieved by upregulating the corresponding genes by interaction with antioxidant response elements (AREs) that trancriptionally regulate these genes (Ferguson, 2001; Iqbal *et al.*, 2003).

Glutathione transferase is an abundant cellular enzyme in mammalian tissues. Glutathione transferase is generally viewed as a phase 2 enzyme, primarily involved in the detoxification of electrophilic xenobiotics via catalyzing the formation of GSH–electrophile conjugate (Xie *et al.*, 2001). Several recent studies have also reported that glutathione transferase plays an important role in protecting cells against ROS-mediated injury through catalyzing the decomposition of lipid hydroperoxides generated from oxidative damage of cellular lipid molecules (Xie *et al.*, 2001; Yang *et al.*, 2002). Accordingly, the induction of glutathione transferase by TQ in liver cells may contribute partially to the increased resistance of the TQ-pretreated cells to chemical-elicited toxicity and the neoplastic effects of carcinogens.

Quinone reductase catalyzes the two-electron reduction of polycyclic aromatic hydrocarbons such as quinones to hydroquinone, has been considered to be a detoxification pathway, since the resulting hydroquinone may be conjugated and excreted through mercapturic pathways (Ross *et al.*, 2000). In this context, as mentioned above, quinone reductase may act as an antioxidative enzyme via its ability to maintain the cellular levels of ubiquinol and vitamin E, two important non-protein antioxidants (Ross *et al.*, 2000). The potent induction of quinone reductase after TQ administration would be expected to accelerate detoxication and elimination of carcinogenic metabolites, and thus reduce the incidence of carcinogenesis and may also be involved in the protective effects of TQ on chemical-induced organ toxicity.

In conclusion, this study demonstrates that two endogenous phase 2 enzymes in mice liver can be induced by TQ in a dose-dependent manner and suggest that such an effect may be one of the possible mechanisms of chemoprotective effects associated with TQ in several animal models of chemical carcinogenesis and toxicity. Indeed future studies should give more attention to evaluate the induction of phase 2 enzymes in different animal species, and after different doses, routes and administration period.

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