

Protective effects of *Nigella sativa* oil and thymoquinone against toxicity induced by the anticancer drug cyclophosphamide

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Introduction

The seed of *Nigella sativa* L known as black cumin is a promising medicinal plant component reported to have potent antioxidant effects, and has been used in the Middle East and Far East as a traditional medicine for a wide range of illnesses. Pharmacological properties of the seed have been reported, including anti-inflammatory, anticancer, antifertility, antidiabetic, antimicrobial, antihistaminic and hypotensive effects.^{1–4} The seeds contain more than 30% fixed oil and 0.4–0.45% (w/w) volatile oil, including 18.4–24% thymoquinone (TQ) and 46% of many monoterpenes such as ρ -cymene and α -piene.^{5–8} Much of the biological activity of the seed has been shown to be due to TQ, the active component of the essential oil. The beneficial medicinal effects of *N. sativa* oil (NSO) and TQ have been attributed to their radical scavenging (anti-oxidative) activity^{3,9,10} and its ability to inhibit the production of 5-lipoxygenase products during inflammation.¹¹

The seed oil has been shown to have a potent chemopreventive effect against KBrO₃-induced renal toxicity in rats^{12,13} and to lower blood glucose level in diabetic rats through either extrapancreatic action^{11,14} or via a stimulatory effect on β -cell function,^{15,16} with consequent increase in serum insulin level in both cases. The high antioxidant potential of TQ has been found to reduce nephropathy-related toxicity, including proteinuria and hyperlipidaemia, associated with nephrotic syndrome,¹⁷ gentamicin-induced nephrotoxicity in rats¹⁰ and cyclosporine A-induced injury in rat heart as demonstrated by normalised cardiac histopathology, decreased lipid peroxidation, and improved antioxidant enzyme status and cellular protein oxidation.¹⁸ Likewise, TQ treatment can markedly lowered CCl₄-induced hepatotoxicity through antioxidant mechanisms.¹⁹

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ABSTRACT

Constituents of the *Nigella sativa* seed are reported to possess potent antioxidant effects. Treatment with anticancer drugs such as cyclophosphamide (CTX) is associated with significant toxicity due to over-production of reactive oxygen species, resulting in increased levels of oxidative stress. The aim of this study is to test whether or not *N. sativa* L oil (NSO) or its active ingredient, thymoquinone (TQ), can reduce CTX-induced toxicity. Male albino rats were treated with intraperitoneal administration of phosphate buffered saline (PBS) or 200 mg/Kg CTX followed by intragastric administration of NSO or TQ on alternate days for 12 days. Administration of NSO and TQ was initiated 6 h before or after CTX injection. Twenty-four hours after the last NSO and TQ treatment, blood and liver were harvested to analyse toxicity-related parameters. Treatment with CTX induced significant toxicity as shown by decrease in haemoglobin concentration and increases in blood sugar levels, activities of liver enzymes, bilirubin, urea, creatinine, lipids (triglyceride, cholesterol and low-density lipoprotein (LDL)-cholesterol) and lipid peroxidation in the liver. Treatment with NSO or TQ induced significant reduction in overall toxicity. The antitoxic effects of NSO and TQ were associated with induction of antioxidant mechanisms. These results suggest that administration of NSO or TQ can lower CTX-induced toxicity as shown by an up-regulation of antioxidant mechanisms, indicating a potential clinical application for these agents to minimise the toxic effects of treatment with anticancer drugs.

KEY WORDS: Antioxidants.
Cyclophosphamide.
Nigella sativa.
Ranunculaceae.
Thymoquinone.

Importantly, NSO has a high LD₅₀ value and does not alter hepatic enzyme stability or organ integrity in mice and rats, suggesting a wide margin of safety for its application.²⁰ Similarly, the LD₅₀ value of TQ after intraperitoneal and oral administration has been found to be 10–15 times and 100–150 times, respectively, greater than doses that show anti-inflammatory, antioxidant and anticancer effects.²¹ Thus, it appears that NSO and TQ are relatively safe compounds.

Cyclophosphamide (CTX) is an alkylating agent with remarkable therapeutic activity against a wide range of conditions, including autoimmune disease (e.g., systemic

sclerosis) and cancer.^{22–26} However, CTX treatment causes significant systemic toxicity due to the over-production of reactive oxygen species (ROS) that cause oxidative stress.²⁷ Although CTX-induced toxicity can be reduced by lowering the dose, which can still be effective, for example, in severe lupus nephritis,²⁸ high-dose CTX is required to treat most cancers and refractory autoimmune diseases.²⁹ A potential approach that can interfere with CTX-induced toxicity is to block or lower the induced oxidative stress.

This study aims to test whether or not the provision of these compounds during CTX treatment can significantly lower the associated toxicity.

Materials and methods

Animals

Male albino rats (WISW strain; weight: 123–178 g; age: 2–3 months) were obtained from Theodore Bilharz Research Institute, Cairo, Egypt. They were housed with natural ventilation and illumination in a temperature-controlled animal room (20–23°C) and provided with food and water *ad libitum*. Animals were deprived of food 12 h before receiving treatment. All animals received care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals, National Academy of Sciences, published by the National Institutes of Health.

Chemicals

Cyclophosphamide (>98%, Cycram; Tanta Cancer Centre, Egypt) was dissolved in sterile water for intraperitoneal (i.p.) injection. The NSO (Biopharm, Cairo, Egypt) was stored in the dark to avoid oxidation. The TQ (99%; 2-isopropyl-5-methyl-1,4-benzoquinone; Sigma-Aldrich) was dissolved in dimethyl sulphoxide (DMSO) and then diluted in PBS as required.

Experimental and treatment protocols

Rats ($n=6$ /group) were assigned randomly and treated with: 1) PBS alone (control group); 2) CTX alone; 3) CTX and NSO 6 h before CTX treatment; 4) CTX and TQ 6 h before CTX treatment; 5) CTX and NSO 6 h after CTX treatment; 6) CTX and TQ 6 h after CTX treatment; 7) NSO alone; and 8) TQ alone. Groups 7 and 8 served as controls for groups CTX + NSO and CTX + TQ, respectively. Cyclophosphamide was administered (i.p.) by two injections (200 mg/kg/injection) two days apart.^{30–34} The NSO and TQ were administered through intragastric injection (1 mL/kg and 10 mg/kg, respectively) every other day for 12 days.^{17,20,35}

Preparation of plasma and the liver samples

Twenty-four hours after the last NSO and TQ treatments, rats were deprived of food for 12 h, dispatched using ether, and blood and liver tissue samples were harvested. Blood was collected in heparinised tubes containing 5000 iu/mL heparin sodium. Fresh blood was centrifuged at 3000 rpm for 15 min. Plasma was separated and aliquots were stored at –20°C until required. Liver was perfused with ice-cold PBS and homogenised (20% w/v) in chilled potassium chloride (1.17%) using an electric homogeniser. The homogenates were centrifuged at 800 \times g for 5 min at 4°C. The supernatant was collected and centrifuged at 10,500 \times g for 20 min at 4°C to enrich the post-mitochondrial components.

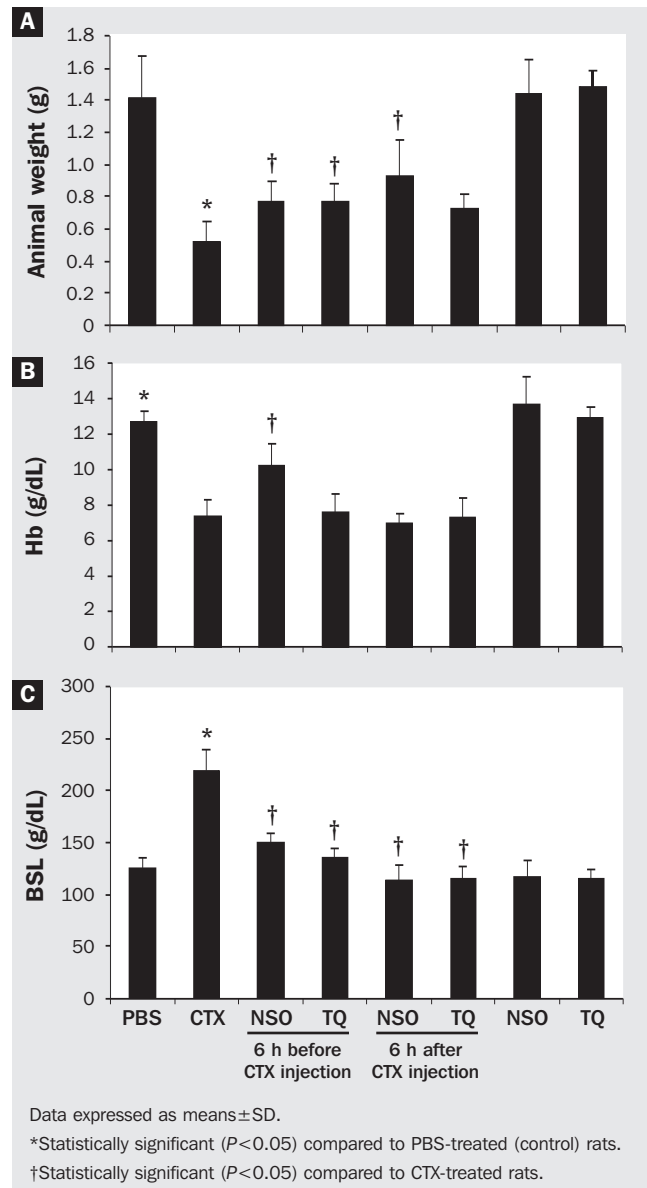


Fig. 1. Effect of oral administration of *Nigella sativa* oil (NSO) or thymoquinone (TQ) on cyclophosphamide (CTX)-induced alteration in the haemoglobin (Hb) and blood sugar level (BSL). Rats ($n=6$ /group) were treated with an i.p. injection of PBS (PBS group), CTX alone (CTX group), CTX plus intragastric injection of NSO (1 mL/kg) or TQ (10 mg/kg) six hours before CTX and then every other day for 12 days, or CTX and intragastric injection of NSO or TQ six hours after CTX treatment and then every other day for a total of 12 days. Additional groups of rats ($n=6$ /group) received intragastric injection of NSO or TQ every other day for a total of 12 days. Twenty-four hours after the last NSO or TQ treatment, rats were weighed (A) and then dispatched with ether. Blood was taken for Hb (B) and BSL (C).

Homeostasis indices

Blood samples were drawn from the lateral tail vein. Blood sugar levels were measured using a blood glucose sensor electrode (Abbott Laboratories, Bedford, UK). The induction of diabetes by CTX was deemed to have occurred when blood glucose exceeded 200 mg/dL.³⁶ Haemoglobin concentration was determined immediately after blood collection as described previously.³⁷

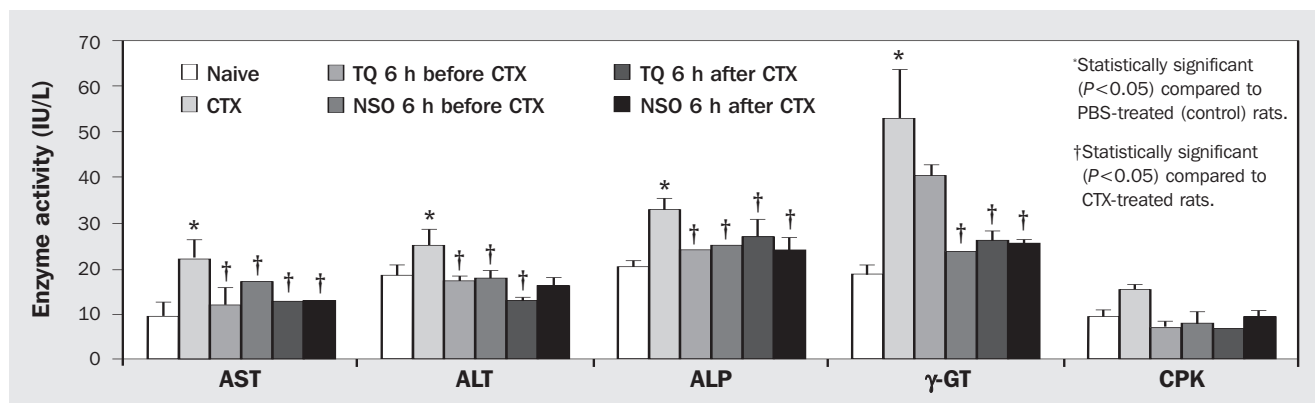


Fig. 2. Effect of oral administration of NSO or TQ on CTX-induced alteration in plasma levels of liver enzymes. Rats ($n=6$ /group) were treated and dispatch as described previously. Levels of AST, ALT, γ -GT and CPK were measured as described in the text.

Assessment of liver function

The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method³⁸ based on determination of oxaloacetate hydrazone and pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. Alkaline phosphatase (ALP) activity was estimated as described previously.³⁹ Plasma γ -glutamyl transferase (γ -GT) activity was measured using the substrate L- γ -glutamyl 4-nitroanilide, in the presence of glycylglycine, as described previously.⁴⁰ Creatine phosphokinase (CPK) activity was determined according to a kinetic method.⁴¹⁻⁴³ Total bilirubin concentration was determined as described previously, where bilirubin is coupled with diazotised sulphanic acid to give an azo dye measured at 578 nm. Plasma urea was estimated at 580 nm by an enzymatic reaction.⁴⁴ Plasma creatinine concentration was assayed by a kinetic method described previously.⁴⁵

Assessment of plasma lipid profile and lipid peroxidation product

Determination of cholesterol was carried out as previously described.^{46,47} This enzymatic colorimetric test is based on the conversion of cholesterol (by cholesterol esterase and oxidase) to quinoneimine dye, the colour intensity of which is directly proportional to the concentration of cholesterol, and can be determined photometrically at 546 nm. Plasma triglyceride level was estimated by an enzymatic colorimetric test,^{47,48} using a lipoprotein lipase for hydrolysis of triglyceride to glycerol, followed by oxidation by peroxidase to form a red dye (Trinder endpoint), the colour intensity of which is directly proportional to the concentration of triglyceride, and can be determined at 546 nm. Plasma LDL concentration was measured at 546 nm by precipitation with heparin (0.68 g/L, corresponding to 100.00 iu/L) using a commercially available kit. Malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS), as described previously.^{49,50}

Estimation of reduced glutathione and catalase

Reduced glutathione (GSH) levels in liver were assayed as described previously⁵¹ and expressed as $\mu\text{mol/L}$ GSH consumed/g liver tissue. Catalase (CAT) activity was assayed as described previously¹⁷ and calculated as units/g tissue.

Statistical analysis

The results were analysed by descriptive statistics and were expressed as mean \pm standard deviation (SD). Inter-group variation was measured by one-way analysis of variance (ANOVA) followed by *t*-test to evaluate the significant differences between the groups. Significance was set at $P \leq 0.05$.

Results

Effect on body weight

Administration of CTX significantly reduced the growth rate of the PBS-treated control rats by approximately 64% (Fig. 1A). Administration of NSO or TQ to CTX-treated rats induced a slight but significant ($P < 0.05$) reduction in body weight (approximately 38% and 71%, respectively; Fig. 1A). Treatment of control rats with either NSO or TQ alone had no effect on growth rate.

Effect on haemoglobin and blood sugar level

Figure 1B shows the effect of treatment with CTX alone or in combination with NSO or TQ on haemoglobin level. Treatment with CTX alone induced a significant ($P < 0.05$) 41% reduction in haemoglobin from 12 ± 0.56 g/dL in PBS-treated rats to 7 ± 0.88 g/dL. Approximately 37% of this effect was seen when NSO was administered 6 h before CTX treatment, but not with administration of TQ. Treatment with NSO or TQ 6 h after CTX treatment had no effect on CTX-induced alteration in haemoglobin level. Treatment of PBS-treated rats with either NSO or TQ had no effect on haemoglobin level.

Figure 1C shows the influence of treatment with CTX alone or in combination with NSO or TQ on blood sugar level. This resulted in a significant ($P < 0.05$) increase in blood sugar level from 126 ± 9 mg/dL to 219 ± 19 mg/dL. Pretreatment with NSO or TQ resulted in a 38% and 31% reduction in the effect of CTX on blood sugar level, respectively. However, initiation of treatment 6 h after CTX treatment produced 48% and 47% improvement, respectively. These effects were significant compared to CTX-treated rats ($P < 0.05$). Treatment of PBS-treated rats with NSO or TQ alone produced 8% and 9% decrease in the blood sugar level, respectively.

Effect on liver function

As shown in Figure 2, CTX treatment resulted in a significant ($P<0.05$) increase (135%) in AST level in the plasma, from 9.5 ± 2.2 IU/L to 22.33 ± 3.01 IU/L ($P<0.05$). It also induced a significant ($P<0.05$) increase (32%) in ALT level, from 18.8 ± 2.23 IU/L to 24.8 ± 3.8 IU/L ($P<0.05$). Pretreatment with NSO and TQ 6 h before CTX treatment induced 46% and 23% recovery of AST activity, respectively, and 31% and 28% recovery of ALT activity, respectively. When administration was initiated 6 h after CTX treatment, NSO and TQ induced comparable (42%) recovery of AST activity, and 48% and 34% recovery of ALT activity, respectively. These effects on AST and ALT were significant compared to CTX-treated rats ($P<0.05$). Plasma AST and ALT activities in rats treated with NSO and TQ alone were similar to levels in PBS-treated rats.

As shown in Figure 2, treatment with CTX resulted in a significant ($P<0.05$) increase (63%) in the plasma ALP level, from 20.33 ± 1.37 IU/L to 33.17 ± 2.48 IU/L. Pretreatment with NSO and TQ 6 h before CTX treatment produced 27% and 24% improvement, respectively. When administration was initiated 6 h after CTX treatment, it induced 18% and 28% improvement, respectively. Importantly, treatment of rats with NSO or TQ alone produced 19% and 29% increases in ALP, respectively. Cyclophosphamide alone led to an approximate three-fold increase (181%) in plasma γ -GT level, from 18.81 ± 1.92 IU/L to 52.92 ± 18.81 IU/L. Pretreatment with NSO or TQ 6 h before CTX treatment induced 25% and 55% increase, respectively. However, administration induced a similar 51% increase when initiated 6 h after CTX treatment. These effects on ALP and γ -GT were significant when compared to CTX-treated rats ($P<0.05$). Treatment of PBS-treated rats with NSO or TQ alone resulted in 30% and 14% elevation in plasma γ -GT level, respectively (Fig. 2).

As shown in Figure 2, CTX treatment induced a significant ($P<0.05$) increase (approximately 62%) in plasma CPK activity, from 9.44 ± 1.48 IU/L to 15.29 ± 1.39 IU/L. Treatment with NSO or TQ showed a tendency to recover the CPK activity to its normal value. Pretreatment with NSO and TQ 6 h before CTX treatment induced an approximate 53% and 47% increase, respectively. Their administration 6 h after CTX treatment induced an approximate 56% and 38% increase in CPK level up-regulated by CTX treatment, respectively. These effects were significant compared to CTX-treated rats ($P<0.05$). Treatment of PBS-treated rats with NSO or TQ alone did not induce any change in plasma CPK level.

Effect on bilirubin, urea and creatinine

Cyclophosphamide treatment induced significant increase ($P<0.05$, approximately 103%) in plasma urea (Fig. 3A), from 32.96 mg/dL ± 2.22 to 66.92 ± 5.16 mg/dL. Pretreatment with NSO or TQ 6 h before CTX treatment induced 43% and 37% reduction in urea level. When administration was initiated 6 h after CTX treatment, it resulted in a 48% and 26% change in urea level, respectively. These effects were significant compared to CTX-treated rats ($P<0.05$). Administration of PBS-treated rats with TQ, but not NSO, significantly ($P<0.05$) increased urea level by 81%.

Cyclophosphamide treatment induced 219% increase in the plasma creatinine level, from 0.57 ± 0.08 mg/dL to 1.82 ± 0.15 mg/dL (Fig. 3B). Pretreatment with NSO and TQ 6 h before CTX induced an approximate 53% and 20% reduction in plasma creatinine level, and induced a similar

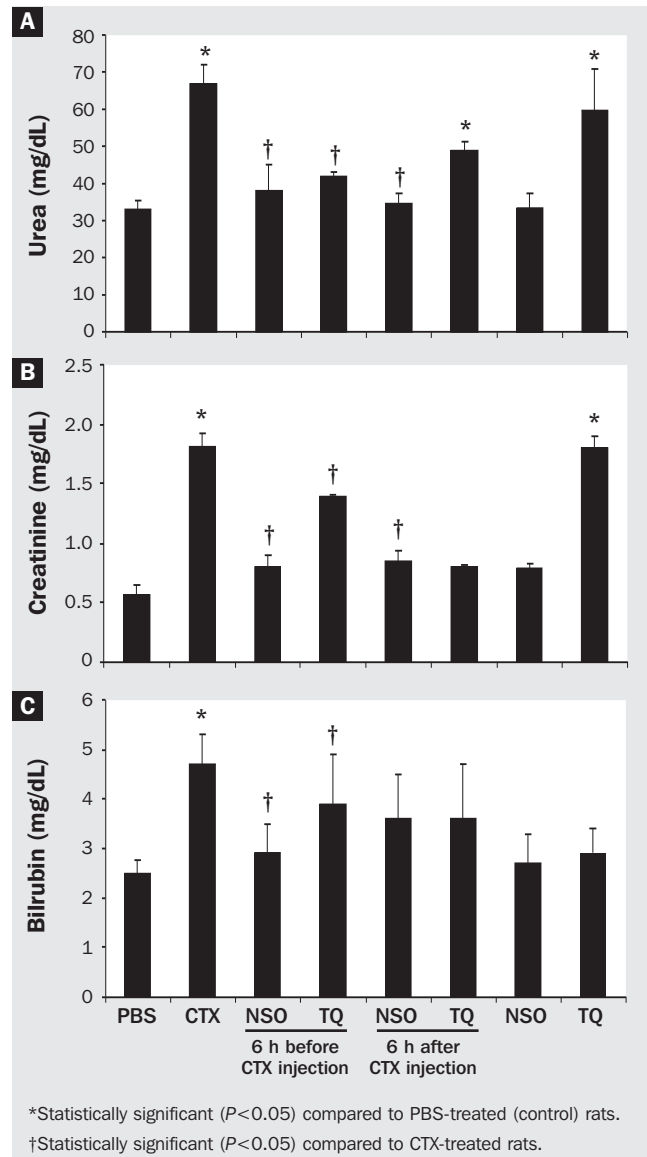


Fig. 3. Effect of oral administration of NSO or TQ on CTX-induced alteration in levels of urea, creatinine and bilirubin. Rats ($n=6$ /group) were treated and dispatched as described previously. Levels of urea (A), creatinine (B) and bilirubin (C) were measured as described in the text.

reduction (56%) when administered 6 h after CTX treatment. These effects were significant compared to CTX-treated rats ($P<0.05$). Administration of PBS-treated rats with TQ, but not NSO, significantly ($P<0.05$) increased creatinine level by 44%.

Treatment with CTX induced a significant ($P>0.05$) increase (approximately 86%) in plasma bilirubin level (Fig. 3C), from 2.54 ± 0.56 mg/dL to 4.68 ± 0.56 mg/dL. Pretreatment with NSO and TQ 6 h before CTX treatment induced 38% and 15% reduction in bilirubin level, respectively. Initiation of NSO or TQ administration 6 h after CTX treatment induced comparable (38%) reduction in bilirubin. These effects on bilirubin level were significant compared to CTX-treated rats ($P<0.05$). Administration of NSO or TQ had no significant effect on plasma bilirubin level.

Effect on plasma lipids

Treatment of rats with CTX resulted in an approximate 100% increase ($P < 0.05$) in plasma triglyceride level, from 27.79 ± 2.1 mg/dL to 56 ± 0.11 mg/dL (Fig. 4A). Pretreatment with NSO or TQ 6 h before CTX treatment induced reduction in the CTX-induced effect on triglyceride by approximately 21% and 23%, respectively. Administration 6 h after CTX treatment induced 20% and 19% reduction, respectively. These effects were significant compared to CTX-treated rats ($P < 0.05$). Their administration in the absence of CTX had no effect on triglyceride levels.

Cyclophosphamide treatment resulted in an approximate three-fold increase (219%, $P < 0.05$) in the plasma cholesterol level, from 27.59 ± 2.54 mg/dL to 87.95 ± 3.49 mg/dL (Fig. 4B). Pretreatment with NSO or TQ 6 h before CTX treatment lowered this effect of CTX by 49%. However, when their administration was initiated 6 h after CTX treatment, it induced 59% and 32% recovery of the CTX-induced changes, respectively. Administration of NSO or TQ alone had no effect on cholesterol level. Similar to its effect on triglyceride and cholesterol, CTX treatment resulted in a significant ($P < 0.05$) increase in plasma LDL level by about 35%, from 76.13 ± 9.57 mg/dL to 102.0 ± 17.62 mg/dL (Fig. 4C). Pretreatment with NSO and TQ 6 h before CTX treatment induced an approximate 73% and 61% recovery, respectively, in LDL level, and an approximate 66% and 59% recovery, respectively, when administration was initiated 6 h after CTX treatment. These effects on cholesterol and LDL were significant compared to CTX-treated rats ($P < 0.05$). Administration of NSO or TQ alone, however, had no significant impact on plasma LDL level.

Effect on hepatic lipid peroxidation

Figure 5 summarises the impact of CTX on lipid peroxidation before and after treatment with NSO or TQ. Figure 5A shows the changes in GSH level. Treatment with CTX significantly ($P < 0.05$) decreased (approximately 69%) hepatic GSH level, from 1227.22 ± 24.63 μ mol/g liver tissue in the PBS-treated rats to 369.07 ± 27.6 μ mol/g. Pretreatment with NSO or TQ resulted in 85% and 134% improvement in GSH level, respectively, and 59% and 132%, respectively, when administration was initiated 6 h after CTX treatment. Treatment of PBS-treated rats with NSO or TQ had no effect on GSH content.

Figure 5B shows the changes in CAT level. Cyclophosphamide treatment induced a 21% decrease in CAT activity in liver tissue. The CTX-treated rats showed 66.95 ± 2.62 units/g tissue, compared to 52.67 ± 2.04 units/g tissue in the PBS-treated rats. Treatment with NSO or TQ before or after CTX treatment had no significant impact on the effect of CTX on CAT activity.

Figure 5C shows the changes in MDA level. Cyclophosphamide treatment caused a marked rise (150%) in TBARS in the liver, measured as MDA (nmol/g tissue). Treatment with NSO or TQ 6 h before or after CTX induced similar (approximately 60%) effect on the CTX-induced increase in MDA. These effects on plasma GSH, CAT and MDA levels were significant compared to CTX-treated rats ($P < 0.05$). Treatment of the PBS-treated rats with either NSO or TQ induced slight but significant ($P < 0.5$) decrease in lipid peroxidation (by 16% and 17%, respectively) of MDA level.

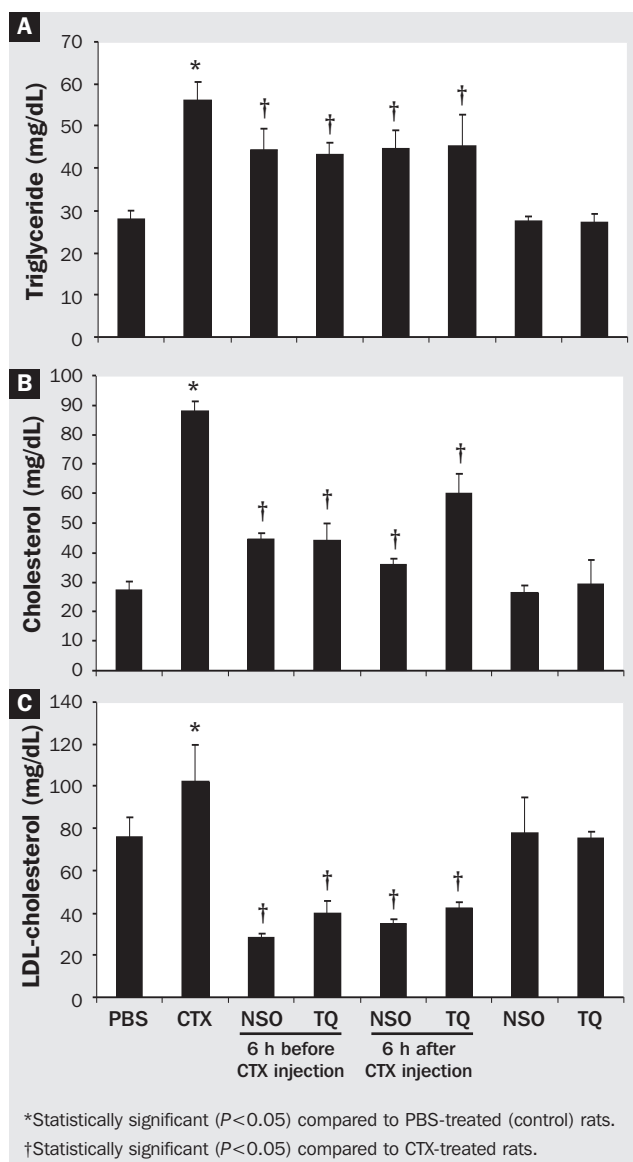


Fig. 4. Effect of oral administration of NSO or TQ on CTX-induced alteration in plasma lipid profile. Rats ($n=6$ /group) were treated and described previously. Levels of triglycerides (A), cholesterol (B) and LDL-cholesterol (C) were measured in liver homogenate as described in the text.

Discussion

N. sativa oil and its active ingredient, TQ, have been characterised as antioxidant and anti-inflammatory agents in different experimental settings.¹ Accordingly, it was hypothesised that treatment with these agents might lower or prevent CTX-induced toxicity. This was investigated in the present study by evaluating the changes in haemoglobin, blood sugar, liver function, serum lipid profile and hepatic lipid peroxidation. The results revealed that co-administration of NSO or TQ with CTX treatment can markedly limit its toxicity through the induction of the antioxidant mediators.

Cyclophosphamide therapy is reported to induce diabetes through widespread destruction of non-renewable β -cells, resulting in hyperglycaemia.⁵²⁻⁵⁴ Cyclophosphamide-

induced β -cell destruction and diabetes has been reported to be associated with the induction of certain pro-inflammatory cytokine (e.g., TNF α and IL-1 β) production by intra-islet macrophages; higher levels of corticosterone caused by β -cell destruction and elevated lipolysis;⁵⁵ up-regulation in expression of the apoptotic molecules Fas and FasL; and up-regulation in expression of the inducible nitrous oxide (NO) synthetase.⁵⁵⁻⁵⁷ Cyclophosphamide-induced diabetes was reversed after treatment with either NSO or TQ.

Several studies also report appreciable hypoglycaemic effects of *N. sativa* and its active ingredients. For instance, treatment with an extract of *N. sativa* seeds⁵⁸ or the active ingredients nigellone and TQ¹⁴ showed antidiabetic activity in streptozotocin-induced diabetes through extrapancreatic and insulenic activities,^{15,59-61} and activation of the cell signalling molecules MAPK and PKB.⁶² Administration of NSO is also able to prevent the chronic increase in insulin level induced after treatment with highly active antiretroviral therapy (HAART).⁶³ The hypoglycaemic effect of NSO has been suggested to be due to its ability to decrease hepatic gluconeogenesis,¹⁶ preserve pancreatic β -cell integrity,⁹ induce lipid peroxidation and increase antioxidant defence system activity.⁶⁴

Toxicity related to anticancer drugs is usually associated with significant hepatotoxicity and nephrotoxicity, due to the alteration in ALT, ALP, lipid peroxidation, urea, creatinine and bilirubin. The data presented here show that CTX treatment is associated with dysregulation of liver function, as shown by increases in AST, ALT, γ -GT and CPK levels. However, these effects can be ameliorated after treatment with NSO or TQ, indicating their protective effects against CTX-induced toxicity. These results are consistent with the findings of previous studies.^{19,65-67} These antitoxic effects were associated with significant improvement in the therapeutic index of the anticancer drugs and in the prevention of non-tumour tissues from sustaining chemotherapy-induced damage.

The nephrotoxicity and hepatotoxicity induced by the toxic effects of anticancer drugs is associated with the retention of urea, creatinine and bilirubin.⁶⁸ In line with the data presented here, treatment with *N. sativa* seed extract has been found to limit the increase in serum albumin and urea level induced by aflatoxin,⁶⁹ *Schistosoma mansoni* infection^{70,71} and KBrO₃.¹³ Most interestingly, supplementation of NSO in humans produced a significant increase in serum total proteins, albumin and globulin,⁶ indirectly indicating that it might be an effective antitoxic agent in the clinical setting.

One mechanism underlying the antitoxic effects of NSO and TQ could be attributed to the enhanced antioxidant effects. Oxidative stress requires different lipids (e.g., triglycerides, cholesterol and LDL-cholesterol), which are transported into cells after binding to LDL receptors.⁷² In line with previous studies,^{73,74} the results presented here show that CTX treatment can induce significant increases in plasma levels of triglyceride, cholesterol and LDL-cholesterol. This CTX-induced dysregulation in the lipid profile was reversed by NSO and TQ. Furthermore, treatment of rats with NSO or TQ has been found to lower the doxorubicin- and CCl₄-induced up-regulation of triglycerides, LDL, cholesterol and lipid peroxides in renal and hepatic tissues.^{17,75}

In line with the findings presented here, several *in vitro*

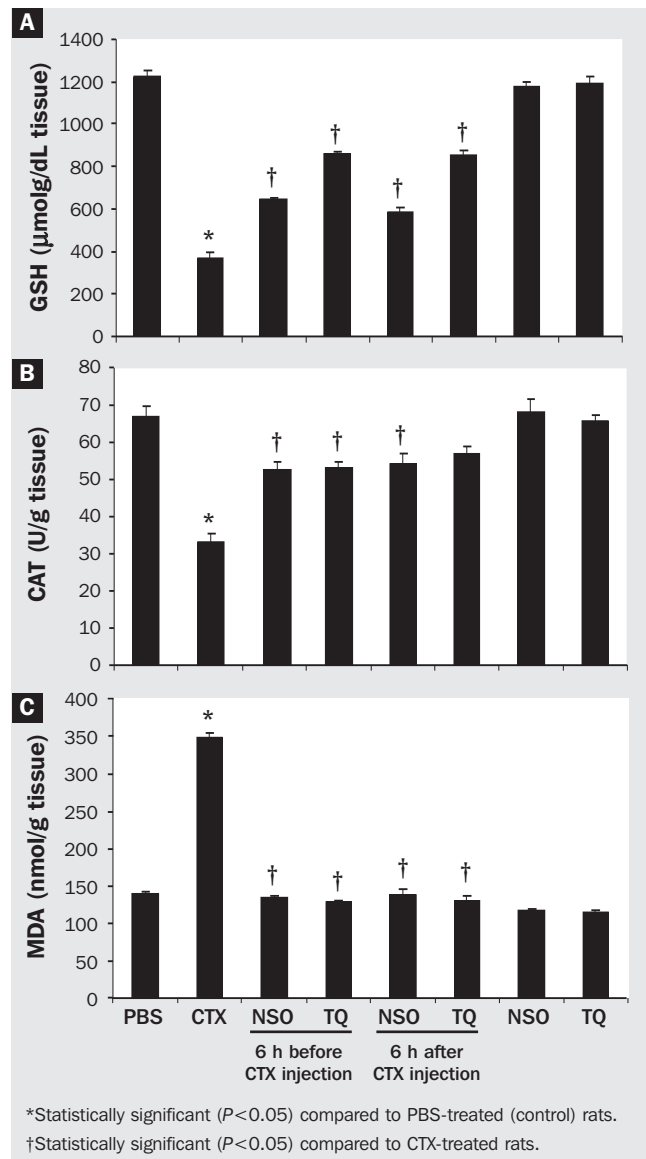


Fig. 5. Effect of oral administration of NSO or TQ on CTX-induced alteration in lipid peroxidation biomarkers. Rats ($n=6$ /group) were treated and dispatched as described previously. Levels of GSH (A), CAT (B) and MDA (C) were measured in the liver homogenate as described in the text.

studies demonstrate potent radical scavenging activity for *N. sativa* components.^{76,77} For example, incubation of macrophages or polymorphonuclear leucocytes with TQ or *N. sativa* seed extract inhibits LPS-induced NO production¹² and protects the cells from superoxide anion radical generated photochemically, biochemically or derived from calcium ionophore – these effects were induced without affecting cell viability.⁷⁸

Other *in vivo* studies also demonstrate the antioxidant effect of NSO and TQ. For example, oral administration of TQ effectively induced increases in quinone reductase and glutathione transferase activities, even in the absence of toxic agents.⁷⁹ Administration of sensitised quinea pigs with NSO or TQ inhibited ROS generation and increased serum levels of the antioxidant enzymes SOD and glutathione.⁸⁰ The *in vivo* antioxidant effects of TQ observed in the present

study extend these *in vitro* and *in vivo* antioxidant effects of NSO and TQ and explain the antitoxic effects of these agents in different models.¹⁻⁴

In conclusion, treatment with anticancer drugs is associated with significant toxicity due to the overproduction of ROS and the associated oxidative stress. The results of the present work indicate that administration of NSO or TQ prior to anticancer chemotherapy can reduce the toxicity through induction of antioxidant mechanisms. In view of the safety and antioxidant properties of NSO and TQ, these results have significant application in the clinical setting to minimise the toxic effects induced during treatment with anticancer drugs. □

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