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RESEARCH ARTICLE

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INFLUENCE OF BLACK SEED (*NIGELLA SATIVA* L) OIL AND ITS ACTIVE INGREDIENT THYMOQUINONE ON CYCLOPHOSPHAMIDE-INDUCED TOXICITY IN MALE ALBINO RATS

ABSTRACT

This study aimed to test whether *Nigella sativa* L oil (NSO) and its purified active ingredient thymoquinone (TQ) can ameliorate the toxicity induced in male albino rats after injection with cyclophosphamide (CP). Thus, rats were orally administered with NSO or TQ before or after single intraperitoneal injection with 200 mg/Kg CP. Injection of the rats with CP induced marked toxicity evidenced by: 1) decreasing the growth rate of the rats and the relative weights of their liver; 2) decreasing the hemoglobin concentration [Hb] and increasing the blood sugar (BS) level; 3) increasing the activities of AST, ALT, ALP, γ-GT, CPK in plasma; 4) increasing the total bilirubin, urea and creatinine coincided with decreasing the albumin level; 5) increasing the concentrations of the plasma lipid profile including; TGs, total CHOL, and LDL cholesterol; 6) increasing lipid peroxidation in the liver indicated by increasing the formation of thiobarbituric acid reactive substance (TBARS), and decreasing the reduced glutathione (GSH) content and catalase activity, and 7) decreasing the plasma levels of T_3 and T_4, which were associated with increasing of TSH. Oral administration of NSO or TQ into CP-injected rats markedly ameliorated the overall CP-induced toxicity evidenced by: 1) the improvement in the body and liver weight; 2) normalization of [Hb] and (BS) levels; 3) amelioration of liver functions; and 4) the recovery of the lipid profile and plasma enzymes. The administration of NSO and TQ was associated with a decrease in the hepatic TBARS and increase in the GSH content, the beneficial effects of NSO and TQ could be attributed to their ability to scavenge or prevent the formation of the oxidative stress species. The results of this study indicate that the oral treatment with NSO or TQ, natural antioxidants, could be considered as adjuvant agents with anti-cancer drug such as CP to lower the associated toxicity.

Key words:

Anti-oxidant, *Nigella sativa*, Thymoquinone, Cyclophosphamide, Liver function, Thyroid function, Oxidative stress.

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INTRODUCTION

Toxicity is a common side effect that associates with administration of several therapeutic drugs used to treat neoplastic and immune disorders. Cyclophosphamide (CP) is an alkylating drug with a remarkable therapeutic activity to inflammatory autoimmune diseases, such as systemic sclerosis, and several cancer diseases (Nadashkevich *et al.*, 2006). Treatment with CP associates with significant systemic toxicity that is attributed mainly to the induction of oxidative stress as a result of the overproduction of reactive oxygen species (ROS) induced by CP metabolites (Mitchell, 2003). Although CP-induced toxicity could be regulated by lowering its administrated dose, which can still be effective for example to treat severe lupus nephritis (Fujinaga *et al.*, 2005), high-dose regimen of CP is required to treat most of cancers and refractory autoimmune diseases (Tsukamoto *et al.*, 2006). Therefore, several studies have focused on identifying effective approaches that can ameliorate, at least in part, some of the CP-induced toxicity. One potential approach that can reduce CP-induced toxicity is through lowering the oxidative effect by administration of potent anti-oxidant compounds. Recent studies pointed out to the efficacy of the application of this anti-oxidant approach. For instance, flavonoids, a group of natural antioxidant products, have been found to be an effective therapeutic regimen ameliorating the bladder damage induced by the oxidant property of CP (Ozcan *et al.*, 2005). Similarly, the prophylactic exposure of a developing mammal to the anti-oxidant indole-3-carbinol has been found to improve the teratogenic effects induced after CP injection (Bailey *et al.*, 2005). Other natural antioxidant compounds including lupeol, ginsenoside Rg3, and squalene have been also found to protect animals from CP-induced cardiotoxicity (Senthilkumar *et al.*, 2006; Sudharsan *et al.*, 2006; Zhang *et al.*, 2006), leading to improvement in the animal survival. In addition, the polysaccharides soy protein (prosobee), and lipoic acid showed cytoprotective effects against CP-induced cytotoxicity in mice (Hui *et al.*, 2006), coinciding with prevention of diabetes (Reddy *et al.*, 2001), testicular

toxicity, (Hui *et al.*, 2006), oxidative stress, and genotoxicity (Selvakumar *et al.*, 2006 a & b).

Nigella sativa L (NS) is a promising medicinal plant that possesses potent antioxidant properties in their seeds known as black cumin or "Habatul-Barakah" (Salem, 2005). NS seeds have long been used in folk medicine in the Middle and Far East as a traditional medicine for a wide range of illnesses. Several pharmacological properties of NS seeds, including anti-inflammatory, anti-cancer, anti-fertility, anti-diabetic, anti-microbial, anti-histaminic, hypotensive, and anti-gastrointestinal problems have been reported (Al-Rowais, 2002; Ali and Blunden, 2003; Goreja, 2003; Salem, 2005). The seeds of NS contain more than 30% fixed oil and 0.4-0.45 % wt/wt volatile oil including 18.4-24% thymoquinone (TQ) and 46% many mono-terpenes such as *p*-cymene and α-piene (El-Kadi and Kandil, 1987). The protective ability of NS oil against toxicity belongs to its radical scavenging (anti-oxidative) activity (Ali and Blunden, 2003; Kanter *et al.*, 2005), to its inhibition of 5-lipoxygenase products during inflammation (El-Dakhakhny *et al.*, 2002a), as well as to its suppression of cell proliferation (Salim and Fukushima, 1995).

Nigella sativa seed oil (NSO) has shown potent chemopreventive effect against $KBrO_3$-induced renal toxicity in rats (Khan *et al.*, 2003). Treatment with NSO led also to a significant reduction in the blood glucose level in diabetic rats either through extrapancreatic action (El-Dakhakhny *et al.*, 2002b) or via a stimulatory effect on β-cell function (Fararh *et al.*, 2002), coinciding with increases in the serum level of insulin in both cases. Much of the biological activity of the NSO has been shown to be due to its active ingredient TQ. The high anti-oxidant potential of TQ has been reported toward nephropathy to protect against proteinuria and hyperlipidemia associated with nephrotic syndrome (Badary *et al.*, 2000). Likewise, TQ treatment markedly lowered the CCl_4-induced hepatotoxicity through induction of antioxidant mechanisms (Nagi *et al.*, 1999). The LD_{50} values of NSO and TQ are high, and the treatment with their pharmacological levels do not alter the stability of hepatic enzyme or organ integrity in mice and rats, suggesting a wide margin of safety for their application in therapeutic purposes (Zaoui *et al.*, 2002b).

The present study aimed to test whether NSO or TQ can ameliorate CP-induced toxicity. The first aim was to establish the toxic effects of CP on the hemoglobin concentration [Hb] and blood sugar (BS) level; lipid peroxidation, plasma enzymes activity, levels of thyroid hormones and pituitary thyrotropin. The second aim was to test the impact of NSO or TQ treatment on these CP-altered parameters. The results reported in this study indicate to the potential application of both NSO and TQ as adjuvant agents during CP-based therapy to lower the associated toxicity.

MATERIALS AND METHODS

Chemicals:

Cyclophosphamide (CP) (Cycram), a water-soluble white powder manufactured by KUP United Douglas Pharm., USA, was kindly provided by Tanta Cancer Centre (Tanta, Egypt). CP was dissolved in a sterile water for i.p. injection. Samples of NSO (Biopharm, Cairo, Egypt) and TQ (2-isopropyl-5-methyl-1,4-benzoquinone, 99% purity; Sigma- Aldrich Co., USA) were kindly gifted by Dr. Mohamed L. Salem, Department of Surgery, Medical University of South Carolina, USA. NSO was stored tightly in dark to avoid the oxidation of the oil. TQ was dissolved in corn oil (10 mg/5 ml) before oral administration.

Animals:

Male albino rats (*Rattus norvegicus*; WISW strain) were purchased from Theodore Bilharz Research Institute (Giza, Egypt). Rats, weighed about (123 -178 g) and aged from 5-6 months, were housed in stainless steel cages with natural ventilation and illumination (dark/light periods) in a temperature-controlled room (20 – 23 ^{o}C). Rats were provided with commercial rodent pellet diet containing 14% protein (Tanta Soap and Oils Co., Egypt), and water *ad libitum.*

Experimental and treatment protocols:

Rats (n = 6~10 rats per group) were administered with either vehicle (control), NSO alone, TQ alone, CP alone, NSO before or after CP injection, and TQ before or after CP injection. NSO was given orally at 1 mL/kg (Zaoui *et al.*, 2002 a & b) and TQ was also given orally at 10 mg/kg TQ (Badary *et al.*, 2000). CP was given as a single intraperitoneal (i.p.) injection at 200 mg/Kg (Hui *et al.*, 2006 and Sudharsan *et al.*, 2006). NSO and TQ treatments were given either 6 hours before or in concomitant with CP injection and they were given every other day for a total of 5 administrations.

During the experimental period, the original weight (W_o) and the final weight (W_f) of the rats were determined to evaluate the rate of growth [(W_f - W_o) / number of experimental days = g/d]. After dissection, livers were excised, blotted on a filter paper, weighed and the relative weight of the liver in relation to the whole body weight was calculated and referred to it as hepatosomatic index [HSI = (liver weight / body weight) X 100].

Preparation of plasma and liver samples:

Twenty-four hours after the last NSO or TQ administrations, all rats were anaesthetized with ether, dissected, and blood and liver samples were harvested. Blood samples were collected in heparinized clean centrifuge tubes (heparin sodium, 5000 IU/ml, USP, Nile Co.,

Egypt). Blood samples were centrifuged at 3000 r.p.m. for 15 minutes, and plasma was separated in aliquots and kept at - 20°C until analysis. The liver was perfused with ice cold saline (0.9% sodium chloride) and homogenized (20% w/v) in potassium chloride (1.17%) using an electric homogenizer. The liver homogenate was centrifuged at 800 g for 5 minutes at 4°C to separate the nuclear debris. The obtained supernatant was then centrifuged at 10,500 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used to assay thiobarbituric acid reactive substances (TBARS). TBARS were assessed by estimating malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) activity.

Haemostatic indices:

Before dissection, blood samples were obtained from the tail vein of concious rats by a needle lancet. Blood sugar (BS) level was measured with blood glucose sensor electrode (Abbott laboratories, Medisince products, Bedford, MA 01730, UK). The induction of diabetes by CP was deemed when the blood glucose readings exceeded 200 mg / dL (Wang *et al.*, 2002). Hemoglobin concentration [Hb] was determined immediately after blood collection (Goyal and Sastry, 1976).

Assessment of enzymes activity and biochemical profiles in the plasma:

The activity of aminotransferases (AST and ALT) was determined according to the conventional method of Reitman and Frankel (1957). The activity of alkaline phosphatase (ALP) was estimated according to the principle of Tietz (1976). The activity of γ-glutamyl transferase (γ-GT) was measured kinetically according to (Szasz and Persijn, 1974). The activity of creatinine phosphokinase (CPK) was determined according to the kinetic method (3 minutes) of Mathieu (1982). The plasma levels of albumin and creatinine were determined according to Tietz (1987) and Henry (1974); bilirubin and urea were estimated using commercial kit reagents.

Assessment of plasma lipid profile:

Determination of total cholesterol (CHOL) level was measured according to Tietz (1995). Plasma triglycerides (TGs) level was estimated according to (Shephard and Whiting 1990). Plasma levels of low density lipoprotein-cholesterol (LDL-CHOL) were also measured at 546 nm by precipitation with heparin 0.68 g/L corresponding to 100.00 IU/L using the kit reagents from Centronic GmbH-Germany.

Estimation of lipid peroxidation biomarkers:

All the investigated indices including; lipid peroxidation, lipid patterns, enzymes activity, and biochemical parameters were measured spectrophotometrically using Spectoronic 21 (Germany). The MDA content, an oxidative stress biomarker, was assayed in the form of TBARS (Okhawa *et al.*, 1979). TBARS were expressed as nmol of MDA per gram tissue. Reduced GSH in the liver, another biomarker of defense system, was assayed as described by Beutler *et al.* (1963). The GSH values were expressed as µmol of GSH consumed/g of liver tissue. The activity of CAT, anti-oxidant, was assessed as described by Claiborne (1985) and calculated as U/g tissue.

Assessment of plasma levels of thyroid hormones and Pituitary thyrotropin:

Plasma levels of the thyroid hormones were assessed by using Immulite 1010 system (F1244 by Cirrus Diagnostics Inc., Loss Angelos, CA 90045, USA). The quantitative measurments of total T_3 and T_4, as well as pituitary thyrotropin (TSH) in the plasma were determined according to Hollander and Shenkman (1974), and Woodhead and Weeks (1985) using immulite kit reagents (LKT_{31}, LKT_{41} and $LKRT_1$), respectively. The measurement is based on solid-phase competitive chemiluminescent enzyme immunoassay which is coated with highly specific monoclonal murine anti- T_3, T_4 and TSH, respectively. However, the specific performance precision values were not more than 10% (≤ 10%) inclu-ding both intra- and inter-assay coefficients.

Statistical analysis:

The results were analyzed and expressed as means ± standard deviations (X ± SD). In all experiments, the results of CP-injected rats and NSO- and TQ-administered rats were statistically tested against the results of untreated control rats. CP-injected rats which administered with either NSO or TQ were tested against CP-injected rats. Change % = value of [(treated rats- control rats/control rats) X100]. The inter-group variation was measured by one-way analysis of variance (ANOVA) followed by *t*-test to evaluate the significant differences between the rats in which the repeated measures of ANOVA indicated values of $P \leq 0.05$. * and ** indicated to the significance ($P \leq 0.05$) mentioned in all the showed Tables throughout the study.

RESULTS

Effect of oral administration with either NSO or TQ on the growth rate (g/day) and hepatosomatic index (HSI) of CP-injected rats:

The impacts of NSO and TQ administration on the CP-associated weight loss, determined by the changes in the growth rate (g/day), and the alteration in the hepatosomatic index (HSI) were illustrated in Table (1). Injection of CP itself significantly reduced the growth rate of the rats by about 63.38% of the vehicle-treated control rats. Administration of NSO or TQ into CP-injected rats ameliorated the CP-induced weight loss by about 38.46% to 71.15%, respectively. The administration of control rats with either NSO or TQ alone had no effect on the growth rate compared to the control rats.

CP injection significantly decreased the value of HSI by about 12.26%.

Table 1: Effect of oral administration with either NSO or TQ on the growth rate (g/day) and hepatosomatic index (HSI) of CP-injected rats

Treatment conditions (No. rats/group)	Treatment time	Growth rate (g/day)		HSI	
		X ± SD	Change (%)	X ± SD	Change (%)
Control (n=6)		1.42 ± 0.26	----	3.59 ± 0.21	----
NSO alone (n=6)		1.44 ± 0.21	1.41	4.44 ± 0.59*	23.91
TQ alone (n=6)		1.48 ± 0.11	4.23	4.46 ± 0.31*	24.55
CP alone (n = 10)		0.52 ± 0.12*	- 63.38	3.15 ± 0.48*	- 12.26
NSO + CP (n=6)	Pre	0.77 ± 0.12**	48.08	3.76 ± 0.33**	19.37
TQ + CP (n=6)	Pre	0.77 ± 0.11**	48.08	3.63 ± 0.48**	15.24
CP + NSO (n=9)	Post	0.93 ± 0.23**	71.15	3.26 ± 0.88	3.49
CP + TQ (n=9)	Post	0.72 ± 0.09**	38.46	3.54 ± 0.93**	12.38

Data are expressed as means ± standard deviations (X ± SD),
n: No. of rats / group.
Pre: pretreatment, Post: post treatment.
* Significant (P ≤ 0.05) compared to the control,
** Significant (P ≤ 0.05) compared to CP-injected group.

Administration of NSO or TQ into CP-injected rats recovered the CP-induced decrease in the HSI, where the ameliorating effect of NSO (19.37%) was slightly higher than the effect of TQ (15.24%). In CP-injected rats, the treatment with TQ, but not NSO, effectively ameliorated the CP-induced decrease in the HSI, inducing about 12.38% recovery of the decrease in the HSI. Interestingly, administration of control rats with either NSO or TQ alone significantly increased the somatic index of the liver by 23.91, and 24.55%, respectively. These results show that treatment of CP-injected rats with NSO or TQ before or after CP can recover the body weight loss and the alteration in the HSI brought about by CP injection.

Effect of oral administration with NSO or TQ on haemoglobin concentration [Hb] and blood sugar [BS] level of CP-injected rats:

Table 2 shows the [Hb] and (BS) level in the CP-injected rats, and the impacts of NSO and TQ treatment protocols on these levels. Injection of rats with CP significantly induced 41.38% reduction in the [Hb]. Administration of NSO, but not TQ, before CP injection significantly induced 37.43% amelioration of the decreasing effects of CP on the [Hb]. Administration of NSO or TQ after CP injection had no effects on the CP-induced alteration in the [Hb]. Similarly, treatment of rats with either NSO or TQ had no effects on the [Hb]. These results indicate that only when given before CP injection, NSO is effective to ameliorate the reducing effects of CP on the [Hb]. CP injection significantly increased the (BS) level by about 73.21% compared to the control rats. Administration of either NSO or TQ before CP injection ameliorated the CP-induced increase in the (BS) levels by 31.48% and 38.09%, respectively.

Table 2: Effect of oral administration with NSO or TQ on hemoglobin concentration [Hb] and blood sugar [BS] level of CP-injected rats.

Treatment conditions (No. rat/group)	Treatment time	[Hb] (g%)		[BS] (mg/dL)	
		X ± SD	Change (%)	X ± SD	Change (%)
Control (n=6)		12.73 ± 0.56	----	126.7 ± 9.29	----
NSO alone (n=6)		13.73 ± 1.55	7.86	116.7 ± 16.35*	- 7.9
TQ alone (n=6)		12.95 ± 0.56	1.73	115.5 ± 8.31*	- 8.82
CP alone (n = 10)		7.4 ± 0.88*	- 41.87	219.4 ± 19.39*	73.21
NSO + CP (n=6)	Pre	10.17 ± 1.29**	37.43	150.3 ± 8.98**	- 31.48
TQ + CP (n=6)	Pre	7.63 ± 1.0	3.11	135.8 ± 8.70**	- 38.09
CP + NSO (n=9)	Post	7.02 ± 0.55	- 5.14	114.0 ± 13.8**	- 48.04
CP + TQ (n=9)	Post	7.27 ± 1.11	- 1.7	115.5 ± 11.23**	- 47.38

Data are expressed as means ± standard deviations (X ± SD),
n: No. of rats / group.
Pre: pretreatment, Post: post treatment.
* Significant (P ≤ 0.05) compared to the control,
** Significant (P ≤ 0.05) compared to CP-injected group.

Administration of NSO or TQ after CP injection ameliorated the CP-induced increase in the (BS) level by 48.04% and 47.38%, respectively. Treatment of rats with NSO or TQ alone slightly, but significantly, reduced the (BS) level by 7.9% and 8.82%, respectively, compared to the control rats. Thus, both NSO and TQ could be considered as effective agents for lowering the increase in the (BS) level caused by CP injection.

Effect of oral administration with NSO or TQ on the activity (IU/L) of the key hepatic enzymes in the plasma of the CP-injected rats:

To evaluate the liver function after CP injection, the activities of the indicator enzymes, including AST, ALT, ALP, γ-GT, and CPK were measured in the plasma as shown in Table (3). CP injection led to a 135.05% increase in the control AST activity, and 31.85% increase in the control ALT activity. Administration of NSO or TQ before CP injection induced 46.26 % and 23.11% recovery of AST, respectively; and 31.13% and 28.36% recovery of ALT activity, respectively. Similarly, immediate administ-ration of NSO or TQ after CP injection induced 41.78% recovery of AST; and 47.79% and 34.43% recovery of ALT activity, respectively. Notably, the AST and ALT activity in all NSO and TQ administered rats were still higher than those in control rats, indicating that these agents did not induce absolute recovery of CP-induced alteration of these enzymes activity. Marginal elevation of AST and ALT activities were noticed in the plasma of the control rats when administered with NSO or TQ. CP injection led to a significant increase (63.16%) in the plasma ALP activity, and the administration of NSO or TQ attempted to significantly lower this effect of CP. Pre-treatment regimen with NSO or TQ induced 27.65 and 24.12%, recovery respectively; and post-treatment regimen led to 18.09% and 27.65%, recovery respectively, of

Table 3: Effect of oral administration with NSO or TQ on the activity (IU/L) of the key hepatic enzymes in the plasma of the CP-injected rats.

Enzyme activity (IU/L)	Control	NSO alone	TQ alone	CP alone	Pre-treatment		Post-treatment	
					NSO+CP	TQ+CP	CP+NSO	CP+TQ
AST (X± SD)	9.5 ± 2.26	10.0 ± 2.68	10.5 ± 2.51	22.33 ± 3.01 *	12.0 ± 4.1 **	17.17± 3.92 **	13.0 ± 4.24 **	13.0 ± 4.24 **
Change (%)	----	5.26	10.53	135.05	- 46.26	- 23.11	- 41.78	- 41.78
ALT (X± SD)	18.84 ± 2.26	19.94 ± 2.97	19.05 ± 2.26	24.86 ± 3.78 *	17.12 ± 1.36 **	17.81 ± 1.75 **	12.98 ± 1.63 **	16.3 ± 1.94 **
Change (%)	----	5.84	1.12	31.95	- 31.13	- 28.36	- 47.79	- 34.43
ALP (X± SD)	20.33 ± 1.37	24.17± 3.87 *	26.17±1.94 *	33.17 ± 2.48 *	24.0 ± 3.95 **	25.17 ± 5.56 **	27.17 ± 3.87 **	24.0 ± 2.76 **
Change (%)	----	18.89	28.73	63.16	- 27.65	- 24.12	- 18.09	- 27.65
γ-GT (X± SD)	18.81 ± 1.92	24.41 ± 2.78 *	21.48 ± 4.78 *	52.92 ± 11.34 *	39.93 ± 2.97 **	23.77 ± 1.97 **	26.06 ± 2.27 **	25.42 ± 1.24 **
Change (%)	----	29.77	14.2	181.34	- 24.55	- 55.08	- 50.76	- 51.97
CPK (X± SD)	9.44 ± 1.48	8.99 ±1.39	9.44 ± 1.48	15.29 ± 1.39 *	7.2 ±1.39 **	8.09 ± 2.41 **	6.75 ± 1.48 **	9.44 ± 1.48 **
Change (%)	----	- 4.77	0.0	61.97	- 52.91	- 47.09	- 55.85	- 38.26

Data are expressed as means ± standard deviations (X ± SD), n: No. of rats / group.* significant (P ≤ 0.05) compared to the control, ** significant (P ≤ 0.05) compared to CP-injected group

the CP-induced increases in the ALP activity. Importantly, administration of NSO or TQ into control rats caused 18.89% and 28.73% elevation of the ALP activity compared to the values in the control rats.

The injection of CP alone led to about 2-fold increase (181.34%) in the plasma γ-GT activity. NSO or TQ administration tended to improve the activity of γ-GT. They ameliorated the CP-induced increase in the γ-GT activity by 24.55 and 55.08%, respectively, when administered before CP injection, and by 50.76% and 51.97%, respectively, when administered after CP injection. Both NSO and TQ caused significant elevations of γ-GT activity by 29.77 and 14.2%, respectively, when given to control rats, compared to untreated control rats. CP injection led to 61.97% increase in the activity of CPK. Administration of NSO or TQ tended to recover the activity of CPK to its control value. Administration of NSO or TQ before CP injection induced about 52.91% and 47.09% recovery, while their administration after CP injection induced about 55.85% and 38.26% recovery. Neither administration of NSO nor TQ into control rats led to any changes in the plasma CPK activity.

Effect of oral administration with either NSO or TQ on the plasma levels of albumin, bilirubin, urea and creatinine of CP-injected rats:

Table 4 shows the levels of albumin and bilirubin; and Table 5 shows the levels of urea and creatinine in all experimental rats. Injection with CP significantly decreased the plasma level of albumin and increased the total level of bilirubin by 18.63 and 85.7%, respectively. Comparing with CP-injected group, rats administered NSO or TQ before or after CP injection showed a marked amelioration in the levels of albumin and bilirubin. Specifically, they recovered the plasma levels of albumin by 8.91% and 10.18%, respectively, when administered before CP injection, and by 7.38% when administered post CP injection.

Table 4: Effect of oral administration with either NSO or TQ on the plasma levels of albumin and bilirubin of CP-injected rats.

Exp groups (Rats, n=6)	Treatment time	Albumin (g/dL)		Total Bilirubin (mg/dL)	
		X ± SD	Change (%)	X ± SD	Change (%)
Control		4.83 ± 0.10	.....	2.52 ± 0.56	.....
NSO alone		4.68 ± 0.23	- 3.11	2.7 ± 0.59	7.14
TQ alone		4.54 ± 0.32	- 6.00	2.88 ± 0.56	14.29
CP alone		3.93 ± 0.38*	- 18.63	4.68 ± 0.56 *	85.7
NSO + CP	Pre	4.28 ± 0.39 **	8.91	2.88 ± 0.56 **	- 38.46
TQ + CP	Pre	4.33 ± 0.35 **	10.18	3.96 ± 1.12	- 15.39
CP + NSO	Post	4.22 ± 0.14 **	7.38	3.6 ± 0.88 **	- 23.08
CP + TQ	Post	4.22 ± 0.35 **	7.38	3.6 ± 1.12	- 23.08

Data are expressed as means ± standard deviations (X ± SD),
n: No. of rats / group.
Pre: pretreatment, Post: post treatment.
* Significant (P ≤ 0.05) compared to the control,
** Significant (P ≤ 0.05) compared to CP-injected group.

Table 5: Effect of oral administration with either NSO or TQ on the plasma levels of urea and creatinine of CP-injected rats.

Treatment conditions (Rats, n=6)	Treatment time	Urea (mg/dL)		Creatinine (mg/dL)	
		X ± SD	Change (%)	X ± SD	Change (%)
Control		32.96 ± 2.22	----	0.570 ± 0.08	----
NSO alone		33.50 ± 4.02 *	1.64	0.79 ± 0.04	38.6
TQ alone		59.83 ± 11.09 *	81.52	1.82 ± 0.15	43.86
CP alone		66.92 ± 5.16 *	103.03	1.82 ± 0.15 *	219.3
NSO + CP	Pre	38.04 ± 7.5 **	- 43.16	0.85 ± 0.09 **	- 53.3
TQ + CP	Pre	42.1 ± 0.98 **	- 37.09	1.46 ± 0.01 **	- 19.78
CP + NSO	Post	34.7 ± 2.77 **	- 48.15	0.85 ± 0.09 **	- 56.3
CP + TQ	Post	49.17 ± 2.27 **	- 26.52	0.80 ± 0.02 **	- 56.04

Data are expressed as means ± standard deviations (X ± SD),
n: No. of rats / group.
Pre: pretreatment, Post: post treatment.
* Significant (P ≤ 0.05) compared to the control,
** Significant (P ≤ 0.05) compared to CP-injected group.

In regard to bilirubin, pre-treatment with NSO or TQ induced 38.46% and 15.39% recovery,

respectively; and their post-treatment regimen induced about 23.08% recovery, respectively. Neither NSO nor TQ administration to control rats showed that any changes in the plasma levels of albumin or bilirubin. CP injection led also to 103.03% and 219.3% increases of the levels of urea and creatinine, respectively. Similar to their ameliorating effects on the albumin and bilirubin, NSO and TQ administrations also ameliorated the levels of urea and creatinine altered by the CP injection. Oral pre-treatment with NSO or TQ induced 43.16% and 53.3% decreases in the urea and creatinine levels, respectively, while TQ pre-treatment led to 37.09 and 19.78% decreases, respectively. Administration of NSO after CP injection led to 48.15% and 56.3% decreases in the urea and creatinine, respectively, and the post-treatment with TQ induced 26.52% and 56.04% decreases in the urea and creatinine. Administration of NSO to control rats was effective to increase only the level of urea, but not the level of creatinine. TQ administration induced 81.52% increase in the urea level and 43.86% in the creatinine levels.

Effect of oral administration with either NSO or TQ on the plasma lipid profile (TGs, total CHOL and LDL-CHOL) of CP-injected rats:

The profile of plasma lipids, including TGs, total CHOL and LDL-CHOL, was analyzed under different treatment conditions (Table 6). It was found that injection of CP resulted in a significant increase in the TGs level by 101.91%. Administration of NSO or TQ before or after CP injections substantially recovered the levels of TGs by 20.83% and 22.8% (for pre-treatment regimen) and 20.14% and 19.07% (for post-treatment regimen), respectively. However, NSO and TQ were able to recover the levels of TGs altered by CP, while neither of them showed any impact on the basal levels of TGs. Injection of CP resulted in about 2-fold increase in the plasma levels of total CHOL, representing about 218.78% of the control value. Eventually, pre- and post-treatment with either NSO or TQ tended to lower the plasma levels of CHOL up-regulated by CP injection. Thus, pre-treatment regimen of NSO and TQ lowered the plasma levels of CHOL by 49.31% and 49.59%; while the post-treatment regimen lowered it by 59.26% and 31.59%, respectively. Administration of NSO or TQ into control rats had no effects on the basal level of total CHOL. By evaluating the plasma levels of LDL-CHOL, CP injection resulted in about 33.98% increase in the basal plasma levels of LDL-CHOL in the control rats. Administration of NSO or TQ before CP injection induced 72.5% and 61.25%, recovery of the LDL-CHOL levels, respectively; while their administration post CP injection gave rise to 65.98% and 58.52% recovery, respectively. Neither NSO nor TQ caused significant impact on the plasma levels of LDL-CHOL.

.Table 6: Effect of oral administration with either NSO or TQ on the plasma lipid profile (TGs, total CHOL and LDL-CHOL) of CP-injected rats

Treatment conditions (Rats, n=6)	Treatment time	Triglycerides (mg / dL)		Total cholesterol (mg / dL)		LDL-cholestrol (mg / dL)	
		X ± SD	Change (%)	X ± SD	Change (%)	X ± SD	Change (%)
Control		27.79 ± 2.1	----	27.59 ± 2.54	----	76.13 ± 9.57	----
NSO alone		27.44 ± 1.19	- 1.26	26.62 ± 2.23 *	- 3.52	78.11 ± 16.54	2.6
TQ alone		27.25 ± 2.08 *	- 1.91	29.08 ± 8.39*	5.4	75.68 ± 2.89	- 0.59
CP alone		56.11 ± 4.42 *	101.91	87.95 ± 3.49*	218.78	102.0 ± 17.62*	33.98
NSO + CP	Pre	44.42 ± 4.98 **	- 20.83	44.58 ± 2.01**	- 49.31	28.05 ± 1.98**	- 72.5
TQ + CP	Pre	43.32 ± 2.93 **	- 22.8	44.34 ± 5.6 **	- 49.59	39.53 ± 6.32**	- 61.25
CP + NSO	Post	44.81 ± 4.42 **	- 20.14	35.84 ± 2.16**	- 59.26	34.7 ± 2.06 **	- 65.98
CP + TQ	Post	45.40 ± 7.64**	- 19.07	59.86 ± 6.97**	- 31.95	42.31 ± 2.68**	- 58.52

Data are expressed as means ± standard deviations (X ± SD), n: No. of rats / group. Pre: pretreatment, Post: post treatment.

* Significant (P ≤ 0.05) compared to the control, ** significant (P ≤ 0.05) compared to CP-injected group.

Table 7: Effect of oral administration with NSO or TQ on lipid per oxidation biomarkers (MDA, GSH and CAT) in CP-injected rats.

Treatment conditions (Rats, n=6)	Treatment time	MDA (nmol / g. tissue)		GSH (μ mol / g. tissue)		CAT (U / g. tissue)	
		X ± SD	Change (%)	X ± SD	Change (%)	X ± SD	Change (%)
Control		139.66 ± 3.3	----	1227.22 ± 24.63	----	66.95 ± 2.62	----
NSO alone		117.2 ± 2.58 *	- 16.08	1180.47 ± 15.28*	- 3.81	68.09 ± 3.26	2.6
TQ alone		115.05 ± 2.7 *	- 17.62	1191.27 ± 36.82*	- 2.93	65.62 ± 1.78	- 1.99
CP alone		349.21 ± 6.77 *	150	369.07 ± 27.6 *	- 69.93	52.67 ± 2.04 *	- 21.33
NSO + CP	Pre	133.67 ± 2.53 **	- 61.72	684.17 ± 7.45 **	85.38	53.16 ± 2.35	0.93
TQ + CP	Pre	129.34 ± 2.39 **	- 62.96	864.6 ± 2.03 **	134.27	52.92 ± 1.66	0.48
CP + NSO	Post	137.85 ± 7.52 **	- 60.53	587.78 ± 14.82 **	59.26	54.14 ± 2.88 **	2.79
CP + TQ	Post	130.95 ± 5.5 **	- 62.5	857.63 ± 15.12 **	132.38	56.79 ± 2.11 **	7.82

Data are expressed as means ± standard deviations (X ± SD), n: No. of rats / group. Pre: pretreatment, Post: post treatment.

* Significant (P ≤ 0.05) compared to the control, ** significant (P ≤ 0.05) compared to CP-injected group.

Table 8: Influence of NSO or TQ administration on the altered levels of the thyroid hormones and pituitary thyrotropin induced by CP injection.

Treatment conditions (Rats, n=6)	Treatment time	T_3 (ng/dL)		T_4 (µg/dL)		TSH (lµU/ml)	
		X ± SD	Change (%)	X ± SD	Change (%)	X ± SD	Change (%)
Control		1.22 ± 0.05	----	4.13 ± 0.08	----	0.138 ± 0.01	----
NSO alone		1.18 ± 0.02 *	13.93	5.36 ± 0.14 *	29.78	0.261 ± 0.01 *	86.43
TQ alone		1.39 ± 0.03	- 3.28	4.34 ± 0.15	5.1	0.204 ± 0.01 *	45.71
CP alone		0.79 ± 0.04 *	- 35.25	3.07 ± 0.06 *	- 25.67	0.290 ± 0.01 *	107.14
NSO + CP	Pre	1.16 ± 0.07 **	46.84	4.14 ± 0.1 **	34.85	0.133 ± 0.01 **	- 54.14
TQ + CP	Pre	1.12 ± 0.02 **	41.77	4.56 ± 0.13 **	48.53	0.111 ± 0.01**	- 61.72
CP + NSO	Post	0.91 ± 0.03 **	15.19	3.42 ± 0.12 **	11.4	0.105 ± 0.01 **	- 63.79
CP + TQ	Post	0.80 ± 0.03	1.27	3.58 ± 0.13 **	16.61	0.105 ± 0.01 **	- 63.79

Data are expressed as means ± standard deviations (X ± SD), n: No. of rats / group. Pre: pretreatment, Post: post treatment.

* Significant (P ≤ 0.05) compared to the control, ** significant (P ≤ 0.05) compared to CP-injected group.

Effect of oral administration with NSO or TQ on lipid peroxidation biomarkers (MDA, GSH and CAT) in CP-injected rats:

Table (7) shows the levels of MDA, GSH, and CAT in all the experimental rats. By analyzing the lipid peroxidation products of TBARS in the liver of CP-injected animals, it was found that CP injection induced striking increases in MDA by about 150% of the level in the control rats. Eventually, both pre- and post-treatment of CP-injected rats with NSO or TQ decreased the CP-induced elevation in the MDA level (60.53% ~ 62.96%, respectively). The administration of the rats with either NSO or TQ induced slight decreases in the lipid peroxidation products by 16.8% and 17.62% of the control level of MDA, respectively. By analyzing the hepatic GSH, it was found that CP injection markedly decreased its level (69.63%) compared with the control rats. Oral administration of NSO or TQ to CP-injected induced significant amelioration of CP-induced depletion of GSH. NSO or TQ administration induced an increase by 85.38% and 134.27% when they were administered before CP injection; and 59.26% and 132.38% when administered immediately after CP injection (Table 7). Treatment of rats with either NSO or TQ associated with slight, decreases in the liver GSH content. CP injection decreased the CAT activity in the liver by 21.33% of its control value. Administration of NSO or TQ either before or after CP injection had no impact on CAT activity. Similarly, treatment of the rats with either NSO or TQ did not change the basal level of CAT activity.

Influence of NSO or TQ administration on the altered levels of the thyroid hormones and pituitary thyrotropin induced by CP injection:

The influence of NSO and TQ on the CP-induced alteration in the levels of thyroid and thyrotrophic hormones is shown in Table (8). CP injection significantly decreased the plasma levels of T_3 by 35.25% and the T_4 level by 25.67%. Oral administration of NSO or TQ before CP injection induced about 46.84% and 41.77%, respectively, amelioration of the reduced levels of T_3, whilst their administration after CP injection had no effects. The administration of NSO or TQ before CP injection induced about 34.85% and 48.53%, respectively, amelioration of the CP-induced reduction in the levels of T_4, whereas their administration after CP injection induced 11.4% and 16.61%, respectively. Administration of either NSO or TQ alone into control rats had no significant effects on the plasma levels of T_3 or T_4; only NSO administration induced about 13.93% and 29.78% increase in the plasma levels of T_3 and T_4 compared to the control rats, respectively. CP injection induced >1-fold (107.14%) significant increase in the plasma level of TSH. Oral administration of either NSO or TQ before CP injection significantly decreased (by 54.14% and 61.72%, respectively) the plasma levels of TSH. Administration of NSO or TQ after CP injection led to 63.79% amelioration of the reduced levels of TSH induced by CP injection. Importantly, administration of NSO or TQ into healthy rats significantly increased the secretion of pituitary TSH by 86.43% and 45.71%, respectively.

DISCUSSION

In the present study, CP induced a marked decrease in the body weight. Administration of NSO or TQ before or after the CP injection restored the reduced weights to reach values close to the normal, where NSO showed higher effects than TQ. Previous studies reported that rats treated with NSO showed increases in their body weight, which was suggested to be due to an increase in the fat anabolism in muscles and around internal organs (Mustafa *et al.,* 1995; Taha and El-Din, 1997; El-Bolkiny *et al.,* 2003).

Another indication of the CP-associated toxicity found in this study was its decreasing effect on the [Hb]. Administration of either NSO or TQ after CP injection had no effects on the alteration of [Hb] induced by CP; however, when administered before CP injection NSO significantly ameliorated the [Hb]. In line with the ameliorating effects of NSO on the [Hb] found in the present study, oral administration of rats with fixed NSO induced decrease in the number of platelets compared to control

values, whilst induced 6.4% and 17.4% in the haematocrit and [Hb] values (Zaoui *et al.*, 2002a; Al-Jishi and Abuo Hozaifa, 2003).

CP has been established to induce diabetes through causing a widespread dispersion of non-renewable destructed β-cells with pronounced hyperglycemia and higher plasma glycated albumin (Kay *et al.,* 1991; Mizuno *et al.,* 1993; Augstein *et al.*, 1998). Consistent with these studies, the results of the present study showed also that CP injection induced diabetes evidenced by the rise in the (BS) level. Production of high levels of the inducible nitric oxide synthase (NOS), which mediates β-cell destruction, is a mechanism underlying the induction of diabetes by CP (Wang *et al.*, 2002; Redd *et al.*, 2002). Another potential mechanism is the induction of higher levels of corticosterone that mediate β-cell destruction associated with elevated lipolysis and as a consequence higher plasma levels of glycerol and free fatty acids (Wang *et al.,* 2002). However, it has been found that CP-induced diabetes in non-obese diabetic mouse could be prevented by oral administration with anti-oxidant compounds such as nicotinamide or prosobee (Soy protein) (Reddy *et al.*, 2001). Similarly, it was found in the present study that CP-induced diabetes was markedly ameliorated after administration with NSO or TQ evidenced by the decrease in the (BS) level. In line with these results, Zaoui *et al.* (2002a) found that treatment of diabetic rats with fixed NSO decreased the blood glucose levels by 16.5% compared to the control values. Furthermore, in streptozotocin diabetic rats, treatment with NSO, nigellone, or TQ has been reported to express anti-diabetic effects (El-Dakhakhny *et al.*, 2002b). The (BS) level lowering effect of NSO, nigellone, and TQ, however, was not paralleled by a stimulation of insulin release in the presence of exogenous addition of NSO, nigellone or TQ, indicating that the hypoglycemic effect of NSO and these agents might be mediated by extrapancreatic actions rather than by stimulated insulin release. These results support the traditional use of *N. sativa* as a treatment of the hyperglycaemia, and related abnormalities.

Dysfunction of the liver is a major indication to systemic toxicity. The data presented herein revealed that CP injection was associated with alteration of the steady-state activities of AST, ALT, γ-GT and CPK. These alterations were corrected after oral administration of NSO or TQ given before or after CP injection. These results are in consistence with the findings of previous studies, which demonstrated that the administration of either the extract of the black seeds or its oil to rats induced anti-toxic effects associated with decreases in γ-GT, AST and ALT activity (Ali and Blunden 2003, Mahmoud *et al.*, 2002; Khan *et al.*, 2003). A recent study has shown that TQ has promising effects through its combination with clinically used anti-cancer drugs leading to improvement in their therapeutic index and prevention of non-tumor tissues from sustaining chemotherapy-induced damage (Gali-Muhtasib *et al.*, 2006). NS extract and TQ also ameliorated the altered serum activities of ALP, LD, MDH, AST and ALT induced by cisplatin, CCl4-induced, ert-butyl hydropero-xide, and D-galactosamine (El-Daly, 1998; Daba and Abdel-Rahman, 1998; Nagi *et al.*, 1999; El-Dakhakhany, 2000). Furthermore, NSO administration to rats with aflatoxin-induced toxcity caused a significant amelioration of the activities of AST, ALT, γ-GT and LDH in serum (Mansour *et al.*, 2001; Abdel-Moneim, 2004).

CP injection caused a decrease in albumin level in the plasma which could be related to a deficiency in albumin synthesis by the liver cells which could be attributed to the albumin loss rather than hepatocytes damage. The present results showed that the treatment with NSO or TQ ameliorated the alteration in the levels of both plasma albumin and bilirubin in the circulation. The high plasma bilirubin concentration may be due to the impairment of the excretory function of the liver, and this may be accompanied by a high plasma ALP activity (Mayne, 1999). An increase in the plasma levels of bilirubin occurs when excessive cell damage exceeds its hepatic excretion and conjugation; thus, the biliary flow is obstructed as a result of excessive haemolysis induced after CP injection. In a recent study, treatment of male rats with *N. Sativa* seed extract was effective to lower the increase in the serum concentration of albumin induced by aflatoxin (Abdel-Moneim, 2004). The beneficial effect of NSO on the serum albumin level was also noticed after supplementation of NS cake protein to the human (Al-Gaby, 1998) and after infection with *Schistosoma mansoni* (Mahmoud *et al.*, 2002; Al-Jishi and Abou Hozaifa, 2003). The results of the present study showed also that CP injection causes retention of waste products such as urea and creatinine in the circulation, which might be a result of the induced nephrotoxicity. The protective effects of NSO and TQ were extended to explain their tendency to stimulate the glomeruli to excrete these wastes. The present findings are in line with the previous studies showing the restorative effect of NSO on $KBrO_3$-mediated renal oxidative stress in rats (Khan *et al.*, 2003) and on the alteration (elevation) of the serum concentration of urea in the aflatoxin-treated rats (Abdel-Moneim, 2004). Accordingly, it has been suggested that potent chemo-preventive effect of NS against renal oxidative stress and various toxicities in experimental animals is likely to be mediated through reversing the enhancement of blood urea nitrogen, serum creatinine and genotoxicity (El-Daly, 1998; El-Sherbeny, 2001; Turkdogan *et al.*, 2001; Iddamaldeniya *et al.*, 2003).

Injection of rats with CP led also to significant increases in the plasma concentrations of TGs, total CHOL, and LDL-cholesterol.

Oral administration of NSO or TQ before or after CP injection ameliorated the deregulated levels of these lipids. It has been suggested that most body cells can synthesize total CHOL but several feedback mechanisms prevent its intracellular accumulation. Total CHOL, taken up by LDL receptors, inhibits intracellular total CHOL synthesis and prevents further uptake by reducing the rate of synthesis of LDL receptors, and consequently plasma level of total CHOL is raised (Mayne, 1999). Similar to our results, Badary *et al*. (2000) and El-Dakhakhny *et al.* (2000) investigated the effect of NSO and TQ on the nephropathy / hepatotoxicity and oxidative stress induced in rats by doxorubicin or by CCl_4, showed that oral treatment of rats with NSO or TQ lowered serum hyperlipidemia including TGs, LDL, total CHOL and lipid peroxides in both renal and hepatic tissues. These results show the medical significance of both *N. sativa* and TQ as a potential treatment of the dyslipidemia, and related abnormalities induced by ROS-lipid peroxidation.

The toxicity associated with the treatment with several chemotherapeutic drugs has been attributed to the induction of the oxidative stress pathways and the induction of high levels of ROS. ROS is known to be continuously produced during normal physiologic events, and removed by antioxidant defense mechanism. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules (Suzuki *et al.*, 1998). Sudharsan *et al.* (2006) showed that the injection of rats with a single dose of CP caused significant increases in the levels of lipid peroxides and decreases in the levels of catalase and GSH antioxidants in the heart tissue. Some ROS scavengers or inhibitors have protective effects against lipid per oxidation. In mice, CP significantly depleted the GSH content in liver but the application of captopril (CAP) 1 hour before CP injection repleted the GSH content. It appears that CAP, due to its antioxidant activity and by increasing GSH levels, can modulate the reduced cellular thiol content and bone marrow genotoxicity induced by CP (Hosseinimehr and Karami, 2005).

Similar to the anti-oxidant effects of the above compounds, the obtained results showed that administration of NSO or TQ before or after CP injection ameliorated the CP induced increases in the hepatic lipid per oxidation biomarkers. Specifically, the anti-oxidant potencies of these compounds were evidenced by decreasing the levels of TBARS such as MDA, and increasing GSH content. These results are in agreement with the previous reports showing that *N. sativa* possess anti-oxidant effects (Mabrouk *et al.*, 2002; Ali and Blunden, 2003; Iddamaldeniya *et al.*, 2003; Khan *et al.*, 2003; El-Saleh *et al.,* 2004; Abdel-Moneim, 2004; Kanter *et al.*, 2005). It has been reported also that the treatment of sensitized quinea pigs, with NSO or TQ inhibited ROS generation and lipid peroxidation, while increased the serum levels of hepatic super-oxide dismutase (SOD) and glutathione (Houghton *et al.*, 1995). Furthermore, extracts of *N. sativa* seeds has been reported to induce a decrease in lipid peroxidation associated with an increase in the antioxidant defense system, preventing liver damage in CCl_4-treated rats (Meral *et al.*, 2001 and Kanter *et al.*, 2003). Additionally, *N. sativa* seed extract along with cysteine, vitamine E and *Crocus sativa* protected against cisplatin-induced hepatic and renal toxicity (El-Daly, 1998). Administration of *N. sativa* seed extract alone into aflatoxin-injected rats induced a significant amelioration in the hepatic lipid peroxidation product (TBARS) and hepatic GSH content, hepatic catalase and serum glutathione reductase activities, showing antioxidant potentials of *N. sativa* extract (Abdel-Moneim, 2004). Similar to the anti-oxidant effects of *N. sativa* seed extract and oil, TQ has shown a protective effect against CCl_4-hepatotoxicity in mice via an antioxidant mechanism (Nagi *et al.*, 1999). Moreover, administration of TQ has been reported to induce significant reductions in SOD, catalase and GSH-reductase activities (Mansour *et al.*, 2002), and to show protective effect on the isolated rat hepatocytes against ter-butyl hydroxide toxicity (Daba and Abdel Rahman, 1998). It has been suggested that the strong antioxidant effects of NSO and TQ are mediated through scavenging different free radicals and thus acting mainly as a potent superoxide anion scavenger (Badary *et al.*, 2003; Kanter *et al.,* 2005). Campbell *et al.* (1996) suggested that the antioxidants effect of NSO is mediated by increasing the bioavailability of arachidonic acid, resulting in biosynthesis of the cytoprotective prostaglandins, which has the ability to preserve the cell membrane integrity.

The results of the present study showed also significant thyroid dysfunction associated with CP injection. This dysfunction was evidenced by the marked alteration of the plasma levels of thyroid hormones and the pituitary thyrotropin. It has been postulated that alkylating drugs, such as CP, cause release of molecules including the serine protease inhibitors (α1-anti-trypsin and corticosteroid-binding globulin), which have high affinity to bind thyroid hormones and as a consequence deregulation of the thyroid hormones (Benvenga *et al.*, 2002; Chan *et al.*, 2003). In addition, high level of circulating anti-thyroid antibodies has been observed after CP injection, leading to thyroid dysfunction and consequently thyroid lymphoma (Derringer *et al.*, 2000; Skacel *et al.*, 2000; Thieblemont *et al.*, 2002). By evaluating the impact of NSO and TQ on this thyroid dysfunction, it was found that these agents corrected the

hormonal levels, where the effect of NSO was superior to TQ. NSO and TQ administrations also ameliorated the deregulated levels of thyrotropin, indicating to the beneficial effects of these compounds against the CP-induced neurotoxicity. This effect is supported by the previous studies showing that NSO or TQ act at the neural levels (Abdel-Fattah *et al.*, 2000), producing antinociceptive effects through indirect activation of the supra-spinal opioid receptor subtypes. Recent studies showed also that TQ produce anticonvulsant activity, probably through an opioid receptor-mediated effect (Hosseinzadeh *et al.*, 2005).

In conclusion, the overall results presented in this study indicated that CP induced a remarkable toxicity evidenced by reduction in the growth rate of the injected rats and the somatic index of their liver. CP injection led also to alterations in several organ functions evidenced by perturbations of several biochemical parameters. Oral administration of NSO or TQ had beneficial effects toward the homeostatic perturbations caused by CP toxicity. Specifically, these agents expressed anti-oxidative effects, which were reflected by correcting most of the analyzed toxicity related parameters.

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تأثير زيت الحبة السوداء (نيجلا ساتيفا) والثيموكينون أحد مكوناتها النشطة على سمية السيكلوفوسفاميد المستحدثة فى ذكور الجرذان البيضاء

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يهدف البحث إلى دراسة تأثير زيت الحبة السوداء والثيموكينون (أحد المكونات النشطة للزيت) المقدرة على كبح السمية فى ذكور الجرذان البيضاء بعد معاملتها بالسيكلوفوسفاميد. وعليه عوملت الجرذان – عن طريق الفم – بالزيت أو الثيموكينون قبل أو بعد حقنها بجرعة واحدة (200 مجم/كجم) للسيكلوفوسفاميد عن طريق التجويف البريتونى. أشارت النتائج إلى أن:

1) حقن ذكور الجرذان البيضاء مرة واحدة بعقار السيكلوفوسفاميد أدى إلى سمية واضحة تمثلت فى:
   1) تراجع معدل النمو للجرذان ونقص الوزن النسبى لأكبادها
   2) نقص تركيز الهيموجلوبين وزيادة مستوى سكر الدم
   3) زيادة واضحة فى نشاط إنزيمات الكبد فى بلازما الدم ، وأيضاً مستوى كلٍ من البليروبين واليوريا والكرياتينين بينما كان هناك نقص فى مستوى الألبيومين
   4) زيادة مستوى مشتقات الدهون فى بلازما الدم مثل الكوليستيرول الكلى والجليسريدات الثلاثية واللبيوبروتينات منخفضة الكثافة
   5) زيادة أكسدة الدهون فى أنسجة الكبد (زيادة نواتج الأكسدة MDA ونقص فى الجلوتاثيون المختزل ونشاط إنزيم الكاتاليز)
   6) انخفاض مستوى هرمونات الغدة الدرقية وارتفاع مستوى الهرمون المنشط للغدة الدرقية (الثيروتروبين) فى البلازما.
2) العلاج المتكرر بزيت الحبة السوداء أو الثيموكينون سواء قبل أو بعد السيكلوفوسفاميد أدى إلى تحسنٍ ذى دلالةٍ إحصائية للمعايير الفسيولوجية المختلة بالسيكلوفوسفاميد مثل:
   1) تحسن وزن الجسم والكبد
   2) تحسن مستوى سكر الدم وتركيز الهيموجلوبين
   3) تحسن مستوى الدهون ونشاط إنزيمات الأكسدة
   4) إصلاح وظائف الكبد والغدة الدرقية. باعتبار أن المعاملة بزيت الحبة السوداء أو الثيموكينون كان مصحوباً بتقليل نواتج الأكسدة فى الكبد وزيادة الجلوتاثيون المختزل، يمكن تفسير ذلك إلى مقدرة كلٍ منهما لكسح أو منع تكوين شوارد الأكسدة.

تبين نتائج هذا البحث أن المعاملة بمضادات الأكسدة الطبيعية مثل زيت الحبة السوداء أو الثيموكينون من المواد الطبيعية التى لها قدرة على الحماية من آثار السمية الناتجة عن العلاج ببعض العقاقير المضادة للسرطان مثل السيكلوفوسفاميد.

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