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Protective role of *Nigella sativa* oil against reproductive toxicity, hormonal alterations, and oxidative damage induced by chlorpyrifos in male rats

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Abstract

This study is aimed at elucidating the possible protective effects of *Nigella sativa* oil (NSO) in alleviating the toxicity of chlorpyrifos (CPF) on reproductive performance in male rats. Animals were orally administered with NSO (1 ml/kg/day), CPF (20 mg/kg/day), and NSO + CPF every day for 4 weeks. Results showed that CPF decreased spermatid number, sperm count, daily sperm production, and sperm motility while increased dead sperm and abnormal sperm compared with the control. Also the levels of testosterone, thyroxine levels, steroidogenic enzyme 17-ketosteroid reductase, body weight, food intake, and relative weight of reproductive organs were decreased. Thiobarbituric acid reactive substances were increased, while glutathione (GSH) and antioxidant enzymes were decreased in plasma and testes of rats treated with CPF. Histopathological examination of testes showed a decrease in the number of seminiferous tubules, form shrinkage, enlargement of the connective tissue and gametogenic changes in germ cells of rats treated with CPF. NSO alone increased testosterone, semen characteristics, GSH, and antioxidant enzymes and decreased the levels of free radicals. Furthermore, the presence of NSO with CPF alleviates its toxic effects. Our results indicated that NSO can improve semen picture and moderate CPF-induced reproductive toxicity.

Keywords

Reproductive toxicity, chlorpyrifos, *Nigella sativa* oil, free radicals, hormones, histopathological examination

Introduction

Many pesticides are known to impair reproductive competence of males in laboratory, field, clinical, or occupational settings (Figa-Talamanca et al., 2001; Giwercman and Giwercman, 2011; Mantovani, 2006; Mantovani and Maranghi, 2005). Organophosphates (OPs) are among the most commonly used pesticides/insecticides in developing countries including Egypt and Algeria.

Chlorpyrifos (CPF) is one of the most widely used OP insecticides throughout the world in both domestic and agricultural applications (Asperlin, 1994). CPF affects the central and peripheral nervous system by inhibiting the acetylcholinesterase (AChE), which breaks down the neurotransmitter acetylcholine (Ach) in the cholinergic synapses and at neuromuscular junctions. The neurotoxic effects of CPF are related to the ability of its metabolite (CPF-oxon) to bind and irreversibly inhibit AChE. The resulting accumulation of ACh in the synaptic cleft causes overstimulation of the neuronal and muscular cells and thereby disrupting

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cholinergic function, which leads to neurotoxicity, paralysis, and eventually death (Eaton et al., 2008).

Previous studies showed that exposure to CPF caused serious health impacts (Ambali et al., 2011; El Mazoudy et al., 2011; Verma et al., 2007). Gultekin et al. (2001) found that CPF increased oxidative stress in different organs, as evidenced by enhanced levels of thiobarbituric acid reactive substances (TBARS), accompanied by concomitant decrease in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

The oxidative stress is known to be a key factor in several diseases. Recent findings indicated that the toxic manifestations induced by CPF may be associated with the enhanced production of reactive oxygen species (ROS), which cause damage to the various membrane components of the cell, especially the accumulation of lipid peroxidation products in different tissues and organs such as liver, kidney, brain, testis, and in fetus (Kalender et al., 2012; Verma et al., 2007; Saulsbury et al., 2009).

ROS can elicit detrimental effects on sperm, inducing DNA damage being associated with risk of male infertility (Ji et al., 2012). Antioxidants can protect against the effects of oxygen species on sperm quality and reproduction (Kalender et al., 2012; Yousef, 2004, 2010; Yousef and Salama, 2010; Yousef et al., 2003a, 2003b, 2004, 2005, 2006; 2007, 2010).

During the past few years, estimation of free radical generation and antioxidants defense has become an important aspect of investigation in mammals. Previous studies were carried out to evaluate the potential role of antioxidants (some medicinal plants as well as many synthetic and natural antioxidants) for the protection of cells against oxidative damage and reproductive toxicity due to environmental toxins (Kalender et al., 2012; Yousef, 2004, 2010; Yousef and Salama, 2010; Yousef et al., 2003a, 2003b, 2004, 2005, 2006, 2007, 2010). These substances have shown their effectiveness to attenuate the oxidative damage, lipid peroxidation, and toxic effects produced in a wide array of systems, organs, and tissues.

Herbalism is an alternative or folk medicine practice, which refers to the use of a plant's part or its extracts and oil for medicinal purposes. It has increased recently and attracted a tremendous attention of many researchers all over the world. It is noteworthy that in some Asian and African countries, 80% of the population depends on traditional medicine for primary health care (WHO, 2008).

Among the promising plants that are believed to have medicinal and healing properties is *Nigella sativa* (*NS*). It is one of the most commonly used plants, the seeds or oil of which is used to fight diseases and promote health in the Middle East, Asia, and Africa (Sharma et al., 2009).

N. sativa is a spice plant commonly known as black seed, black cumin, or "El Habat el-Sawda"; it belongs to the botanical family of Ranunculaceae that was used from prehistoric times as a flavoring agent in bread and pickles. *N. sativa* oil (NSO) has been found to contain over 100 bioactive molecules among which thymoquinone (TQ; 30–48%), *p*-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), *t*-anethol (1–4%), sesquiterpene longifolene (1–8%), thymohydroquinone, dithymoquinone, and α-pinene are some of the predominant compounds (Burits and Bucar, 2000). The seeds and essential oil of *N. sativa* have been subjected to a range of pharmacological investigations in recent years. Studies have shown a wide spectrum of therapeutic properties due to their antioxidant, digestive, appetite stimulant, laxative, galactogogue, emmenagogue, diuretic, diaphoretic, carminative, analgesic, hepatoprotective, renal protective, insecticide, bronchodilator, immunomodulative, hypotensive, choleretic, antibacterial, antifungal, anthelmintic, anti-inflammatory, antispasmodic, antipyretic, antitumoral, antidiabetic, anti-astmatic, and anti-ulcerogenic activities. Most of these beneficial activities are attributed to the presence of TQ, the major bioactive and antioxidant component (Ait Mbarek et al., 2007; Khader et al., 2009; Yaman and Balikci, 2010).

Although in recent years, the knowledge on the toxic effects of CPF markedly improved, data concerning the reproductive toxicity, testicular dysfunction, changes in hormone levels, antioxidant enzyme activities, and oxidative damage still needs more research. Also the role of NSO against CPF-induced changes in reproductive performance has not been studied so far. This study was undertaken to investigate the protective role of NSO against CPF-induced reproductive toxicity in adult male rats.

Materials and methods

CPF and NSO

CPF was obtained from Vapko Company for pesticides and chemicals (Algeria). NSO with 100% purity was obtained from Jeddah manufactory Company for essential oils (Saudi Arabia).

Animals and experimental design

The local Algerian committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institute of Health (NIH). Thirty-two adult male Wistar rats (weighing approximately 200–220 g) were purchased from Pasteur Institute Kouba (Algeria). The animals were acclimatized for 2 weeks prior to the start of the experiment. The animals were individually housed in plastic cages with sawdust bedding and maintained in an air-conditioned animal house at a controlled temperature ($22 \pm 2^{\circ}C$), relative humidity ($60 \pm 10\%$), and in a photoperiod of 12-h light/12-h dark cycle, with free access to pellet feed and fresh tap water. Then, the animals were randomly allocated into four groups of eight rats each as follows: group I: (C), served as control, was administered orally with 1 ml/kg/day of distilled water. Group II: (CPF), animals were given a dose of CPF at the level of 20 mg/kg/day. Group III: (NSO), rats received 1 ml/kg/day of NSO. Group IV: NSO + CPF, animals were administered with a dose level of 1 ml/kg/day of NSO and then after 30 min, received 20 mg/kg/day of CPF. Animals were treated by gavage with CPF and NSO for 4 weeks.

Body and organ weights

The body weight was recorded weekly throughout the treatment period. At the end of the treatment, animals were anesthetized with ether and killed by decapitation. The target (liver, kidney, and adrenal) and reproductive organs (testes, epididymes, and seminal vesicles) were quickly removed and weighed after they were cleared off from their attachment and their connective tissue; relative organ weights were calculated on the basis of the animal weight at the time of killing. Trunk blood samples were collected from the killed animals and placed immediately on ice. Heparin was used as an anticoagulant, and plasma samples were obtained by centrifugation at 860*g* for 20 min and stored at $-60^{\circ}C$ till measurements.

Determination of plasma and testicular antioxidant enzymes, TBARS, and reduced GSH

The content of reduced glutathione (GSH) was determined according to the method described by Jollow et al. (1974). The specific activity of GPx (EC.1.1.1.9) was measured according to the method described by Chiu et al. (1976). The activity of SOD (EC.1.15.1.1) was determined according to the method described by Misra and Fridovich (1972). TBARS was determined according to the method described by Tappel and Zalkin (1959). The activity of glutathione-*S* transferase (GST; EC. 2.5.1.18) was determined according to the method described by Habig et al. (1974). The specific activity of CAT (EC1.11.1.6) was determined according to the method described by Luck (1974).

Assay of steroidogenic enzymes 17-KSR and 17β-HSD

The testicular tissues were homogenized with a Tekmar model TR-10, West Germany homogenizer in 10-volume 0.25 M sucrose containing 0.05 mM ethylenediaminetetraacetic acid and 5 mM mercaptoethanol, buffered with 0.05 M potassium phosphate (pH 7.4); the homogenate was centrifuged at $4^{\circ}C$ in a cooling centrifuge (Heraeus Christ, West Germany). Aliquots of the resulting supernatant were used for crude preparation of 17-ketosteroid reductase (17-KSR; EC 1.1.1.64) and 17β-hydroxysteroid dehydrogenase (17β-HSD; EC 1.1.1.51) enzymes according to the method described by Katryna and Anita (1980).

Protein estimation

The protein concentration of testicular tissues was determined using Lowry protein assay (Lowry et al., 1951).

Semen characteristics

Sperm count and spermatid number. The left testis and epididymis from each rat were excised. After removal of tunica albuginea, the testis was minced with scissors and homogenized in 10 ml 0.9% sodium chloride (NaCl) containing 0.5% Triton X-100; the homogenate was mixed using a vortex mixer. The number of homogenization-resistant spermatids (SNs) was counted using improved Neubauer haemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, Germany). Results were recorded as mean sperm counts per gram organ.

Daily sperm production (DSP) was calculated by dividing the SNs by 6.1, which is the number of days of the seminiferous cycle in which these spermatids are present in the seminiferous epithelium (Blazak et al., 1993).

The left caudal epididymis was cut into small pieces using a disposable blade in 10 ml of 0.9% NaCl containing 0.5% Triton X-100 and homogenized. Spermatozoa were counted as described above. The

Table 1. Changes in BWG (%), FI (g/rat/day), RLW, RKW, RAW, RTW, REW, and RSW (g/100 g BW) during and after treatment of male rats with CPF, NSO, and/or their combination.[a]

Parameter	Groups			
	Control	CPF	NSO	CPF + NSO
Initial body weight(g)	223.17 ± 5.31	225.78 ± 7.8	221.67 ± 2.42	228.7 ± 8.68
Final body weight(g)	273.09 ± 3.54	193.22 ± 5.97	263.83 ± 3.25	241.6 ± 6.64
BWG	22.40 ± 2.12	−14.41 ± 0.31[b]	19.5 ± 0.87[b,c]	5.68 ± 1.74[b,c,d]
FI	28.06 ± 0.44	25.90 ± 0.46[b]	28.36 ± 0.31[b,c]	27.81 ± 0.46[b,c,d]
RLW	3.273 ± 0.27	5.53 ± 0.332[b]	3.93 ± 0.171[b,c]	3.86 ± 0.289[b,c]
RKW	0.25 ± 0.010	0.44 ± 0.012[b]	0.29 ± 0.012[b,c]	0.31 ± 0.017[b,c,d]
RAW($\times 10^{-2}$)	0.88 ± 0.066	1.38 ± 0.101[b]	1.10 ± 0.050[b,c]	1.13 ± 0.075[b,c]
RTW	0.54 ± 0.022	0.48 ± 0.022[b]	0.77 ± 0.025[b,c]	0.54 ± 0.017[c,d]
REW	0.19 ± 0.010	0.15 ± 0.010[b]	0.22 ± 0.018[b,c]	0.19 ± 0.010[c,d]
RSW	0.18 ± 0.096	0.20 ± 0.080	0.22 ± 0.085	0.17 ± 0.058

BWG: body weight gain; FI: feed intake; RLW: relative weight of liver, RKW: relative weight of kidney; RAW: relative weight of adrenal gland, RTW: relative weight of testis, REW: relative weight of epididymis; RSW: relative weight of seminal vesicles; CPF: chlorpyrifos; NSO: *Nigella sativa* oil.

[a]Values are given as mean ± SD, significance at $p < 0.05$.

[b]Values differ significantly from control group.

[c]Values differ significantly from CPF group.

[d]Values differ significantly from NSO group.

epididymal sperm transit rate (STR) was estimated for each male rat by dividing the epididymal sperm counts (SC) by the DSP (Amann et al., 1976).

Sperm motility and morphology analysis. Sperms were collected as quickly as possible after killing. The right caudal epididymis of each animal was excised and placed in 2 ml of warm Hanks's solution at 37°C. The tissue was cut with a scalpel blade to release sperms and then placed in a 37°C incubator for 15 min prior to determine sperm motility. The suspension was stirred and 20 μl were deposed between a warmed microscope slide and cover slip. Motile and nonmotile sperms were manually counted under microscopic observation at 40× magnification in at least 10 separate and randomly selected fields (Linder et al., 1995; Liobet et al., 1995). The cover slip was removed, and the spermatozoa suspension was allowed to dry in air atmosphere. The sample was stained with 1% eosin Y/5% nigrosin and examined at 40× magnification for viability and morphological abnormalities. Three hundred spermatozoa from different fields were examined for each sample as described previously (Linder et al., 1995; Liobet et al., 1995).

Estimation of plasma hormones. Plasma testosterone and free thyroxine (FT4) concentrations were measured using the enzyme-linked immunosorbent assay kits purchased from DRG Diagnostics (GmbH, Germany). Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were assayed by the method described by Beitens et al. (1976) and Santner et al. (1981), respectively.

Histopathological examinations. Small pieces of testis were fixed in Bouin's fluid, treated with graded alcohol, embedded in paraffin, sectioned to 5 μm thicknesses, and stained with hematoxylin and eosin for observation under light microscope. The histopathological examination was carried out to evaluate the possible changes in the seminiferous tubule level (e.g. atrophy), seminiferous epithelium level (e.g. disorganization, depletion), and germ, Sertoli, and Leydig cells level (e.g. degeneration, retention, and vacuolation).

Statistical analysis. Data are expressed as mean values ± standard deviation and analyzed by Statistical Package for the Social Sciences (SPSS; version 11.0) software for Windows. One-way analysis of variance followed by Tukey's procedure was used to determine differences between the groups. The level of significance (p) was set at 0.05.

Results

Body and organ weights

The effects of CPF, NSO, and their coadministration on the body weight gain (BWG), feed intake, and relative weights of vital and reproductive organs are

Table 2. Effect of CPF, NSO, and/or their combination on semen characteristics of male rats for 4 weeks.[a]

Parameter	Groups			
	Control	CPF	NSO	CPF + NSO
Spermatid number ($\times 10^6$/g testis)	245 ± 20.0	182.86 ± 10.97[b]	304.86 ± 16.10[b,c]	230 ± 11.94[c,d]
SC ($\times 10^6$/g epididymis)	140 ± 8.40	108.14 ± 6.04[b]	220 ± 21.98[b,c]	167.14 ± 11.0[b,c,d]
Daily sperm production ($\times 10^6$/g testis)	40.16 ± 3.50	30.01 ± 1.80[b]	49.98 ± 2.64[b,c]	37.70 ± 1.96[c,d]
Sperm transit rate (days)	3.48 ± 0.34	3.6 ± 0.27	5.40 ± 0.60[b,c]	4.43 ± 0.30[b,c]
Sperm motility (%)	77.66 ± 1.26	48.7 ± 1.35[b]	85.33 ± 1.03[b,c]	70.37 ± 0.85[b,c,d]
Dead sperm (%)	23 ± 0.13	34 ± 0.61[b]	15 ± 0.19[b,c]	25 ± 0.35[b,c,d]
Abnormal sperm (%)	4.16 ± 0.36	8.25 ± 0.44[b]	3.01 ± 0.18[b,c]	4.09 ± 0.27[c,d]

CPF: chlorpyrifos; NSO: *Nigella sativa* oil; SC: sperm count.
[a]Values are given as mean ± SD, significance at $p < 0.05$.
[b]Values differ significantly from control group.
[c]Values differ significantly from CPF group.
[d]Values differ significantly from NSO group.

Table 3. Changes in testosterone (ng/ml), FT4 (pmol/l), LH and FSH, and the activities of 17β-HSD and 17-KSR after treatment of male rats with CPF, NSO, and/or their combination for 4 weeks.[a]

Parameter	Groups			
	Control	CPF	NSO	CPF + NSO
FT4	32.88 ± 2.01	26.38 ± 1.6[b]	28.88 ± 0.40[b,c]	30.76 ± 1.48[b,c,d]
Testosterone	3.9 ± 0.20	1.25 ± 0.03[b]	5.44 ± 0.21[b,c]	3.41 ± 0.22[b,c,d]
LH (mIU/ml)	0.69 ± 0.021	1.19 ± 0.018[b]	0.83 ± 0.015[b,c]	0.75 ± 0.016[b,c,d]
FSH (mIU/ml)	0.59 ± 0.017	0.98 ± 0.014[b]	0.71 ± 0.013[b,c]	0.64 ± 0.016[b,c,d]
17β-HSD (U/min/mg protein)	1.21 ± 0.035	3.21 ± 0.045[b]	1.45 ± 0.028[b,c]	1.42 ± 0.043[b,c]
17-KSR (U/min/mg protein)	14.5 ± 0.18	4.1 ± 0.19[b]	18.2 ± 0.31[b,c]	9.2 ± 0.23[b,c,d]
Protein content (mg/g tissue)	130 ± 2.2	79 ± 3.4[b]	145 ± 3.1[b,c]	115 ± 4.1[b,c,d]

FT4: free thyroxine; LH: luteinizing hormone; FSH: follicle stimulating hormone; 17β-HSD: 17β-hydroxysteroid dehydrogenase; 17-KSR: 17-ketosteroid reductase; CPF: chlorpyrifos, NSO: *Nigella sativa* oil.
[a]Values are given as mean ± SD, significance at $p < 0.05$.
[b]Values differ significantly from control group.
[c]Values differ significantly from CPF group.
[d]Values differ significantly from NSO group.

presented in Table 1. CPF induces a significant reduction in BWG, feed intake, and relative weights of testis (RTW) and epididymis (REW) accompanied by an increase in the relative weight of vital organs (liver, kidney, and adrenal gland). Treatment with NSO alone significantly increased the relative weights of all reproductive organs. The presence of NSO with CPF attenuated the relative weights compared with the control group.

Semen characteristics

The changes in sperm characteristics of rats treated with CPF, NSO, and their combination are summarized in Table 2. CPF significantly reduced spermatid number, SC, DSP, and motility and increased dead and abnormal sperm compared with the control group. The administration of NSO alone caused significant increase in spermatid number and SCs, DSP, and motility and decrease in dead and abnormal sperm, in comparison with the controls. Also the presence of NSO with CPF alleviated its toxicity on semen quality compared with CPF group.

Hormones and steroidogenic enzymes 17-KSR and 17β-HSD

Table 3 represents data of testosterone, FT4, LH, and FSH levels as well as the levels of steroidogenic

Table 4. Changes in the levels of TBARS and GSH, and the activities of the antioxidant enzymes (GPX, CAT, SOD, GST) in plasma and testis after treatment of male rats with CPF, NSO, and/or their combination for 4 weeks.[a]

Parameter	Groups			
	Control	CPF	NSO	CPF + NSO
Plasma				
TBARS (nmol/ml)	1.23 ± 0.052	2.22 ± 0.082[b]	0.79 ± 0.051[b,c]	1.41 ± 0.043[b,c,d]
GSH (U/ml)	4.98 ± 0.14	2.89 ± 0.098[b]	6.38 ± 0.212[b,c]	3.88 ± 0.12[b,c,d]
GPX (U/ml)	9.8 ± 0.22	4.9 ± 0.16[b]	14.5 ± 0.28[b,c]	8.1 ± 0.19[b,c,d]
CAT (U/ml)	49.9 ± 2.12	25.7 ± 1.99[b]	69.9 ± 1.39[b,c]	43.9 ± 2.02[b,c,c]
SOD (U/ml)	2.01 ± 0.045	0.88 ± 0.048[b]	3.12 ± 0.056[b,c]	1.78 ± 0.071[b,c,d]
GST (μmol/h)	0.88 ± 0.017	0.49 ± 0.014[b]	1.49 ± 0.023[b,c]	0.72 ± 0.025[b,c,d]
Testis				
TBARS (nmol/g tissue)	25.3 ± 0.77	58.8 ± 2.11[b]	19.8 ± 0.58[b,c]	32.9 ± 1.55[b,c,d]
GSH (μmol/g tissue)	5.1 ± 0.12	2.9 ± 0.09[b]	7.2 ± 0.17[b,c]	4.2 ± 0.12[b,c,d]
GPX (IU/g tissue)	35.5 ± 0.45	18.1 ± 0.35[b]	44.5 ± 0.39[b,c]	31.4 ± 0.28[b,c,d]
CAT (U/mg protien)	67.2 ± 2.11	39.8 ± 2.01[b]	75.9 ± 2.46[b,c]	59.6 ± 2.21[b,c,d]
SOD (U/mg protien)	14.4 ± 0.32	7.9 ± 0.29[b]	19.9 ± 0.38[b,c]	12.0 ± 0.66[b,c,d]
GST (U/mg protien)	1.88 ± 0.053	0.79 ± 0.063[b]	2.54 ± 0.073[b,c]	1.45 ± 0.065[b,c,d]

TBARS: thiobarbituric acid reactive substances; GSH: glutathione; GPX: glutathione peroxidase; CAT: catalase; SOD: superoxide dismutase; GST: glutathione-*S* transferase; CPF: chlorpyrifos; NSO: *Nigella sativa* oil.

[a]Values are given as mean ± SD, significance at $p < 0.05$.

[b]Values differ significantly from control group.

[c]Values differ significantly from CPF group.

[d]Values differ significantly from NSO group.

enzymes 17-KSR and 17β-HSD of rats treated with CPF, NSO, and their combination for 4 weeks. Rats treated with CPF showed a significant ($p < 0.05$) decrease in testosterone, FT4 levels, and 17-KSR activity, while LH, FSH levels, and 17β-HSD activity were significantly ($p < 0.05$) increased in comparison with untreated animals. NSO alone significantly ($p < 0.05$) increased the level of testosterone, FSH, LH, and17β-HSD. The coadministration of NSO with CPF normalized the changes in testosterone, thyroxine (T4), LH, and FSH levels and 17-KSR and 17β-HSD levels to the normal range of hormonal status in all the biomarkers.

Plasma and testicular antioxidant enzymes, TBARS, and reduced GSH

Table 4 represents changes in the levels of TBARS and GSH and the activities of the antioxidant enzymes (GPx, CAT, SOD, and GST) in plasma and testis after treatment of male rats with CPF and NSO or in combination for 4 weeks. Treatment with CPF caused significant ($p < 0.05$) increase in both plasma and testes TBARS and decrease in GSH, GPX, CAT, SOD, and GST compared with the control group. NSO alone showed significant ($p < 0.05$) decrease in plasma and testes TBARS and increase in GSH, GPX, CAT, SOD, and GST levels compared with the control group. The combination group showed that NSO is capable of increasing the activities of GPx, CAT, SOD, and GST and the level of GSH compared with the CPF group but not reached the control values (Table 4). Also NSO significantly reduced the levels of TBARS compared with the CPF group but not reached the control values (Table 4).

Histopathological examinations of testes

The histological investigations of testes of control (Figures 1(a) and 2(a)) and rats treated with CPF (Figures 1(b) and 2(b)), NSO (Figures 1(c) and 2(c)), and CPF + NSO (Figures 1(d) and 2(d)) are presented in Figures 1 and 2. Testes of CPF-treated rats showed a decrease in the number of seminiferous tubules along by degenerative aspect of seminal epithelium, vacuolization, poor/scarce sperm in lumen of some tubules, enlargement of the connective tissue, and depletion of a variety of cell types. Coadministration of NSO and CPF showed that NSO mitigates and modulates the structural alterations toward normal status.

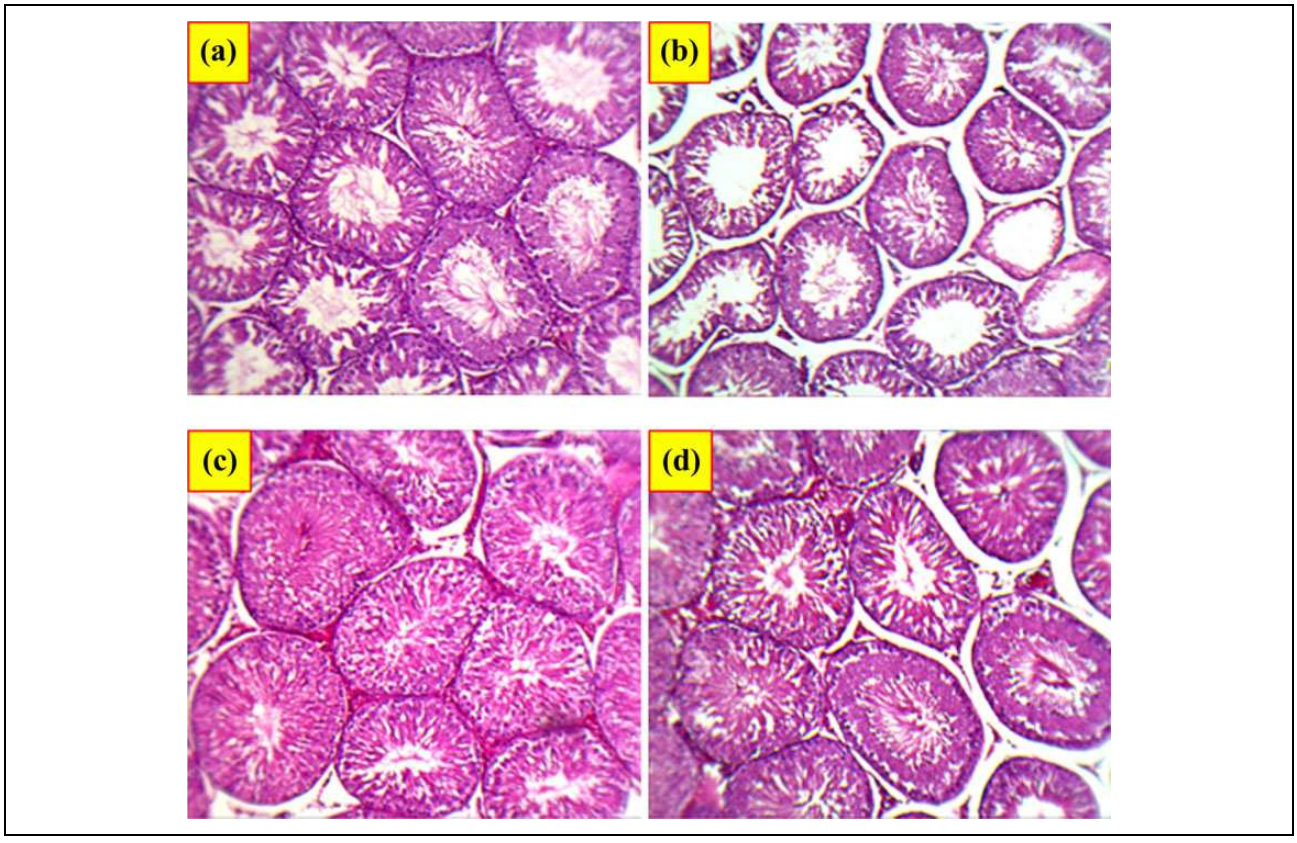

Figure 1. Light microscopic sections of testes from various examined groups: control (a), CPF (b), NSO (c), and coadministration NSO + CPF (d) for 4 weeks. Sections (5 μm) were stained with hematoxylin–eosin (×100). CPF: chlorpyrifos; NSO: *Nigella sativa* oil.

Discussion

Chlorpyrifos

Previous studies were focused on the adverse effects of CPF on the main functional and structural aspects of the most vital organs but not on the reproductive systems, sperm quality, and fertility. Cholinesterase inhibition and oxidative stress are considered as the chief mechanism of action by which these effects may be occurring. Recent studies have identified that CPF induces oxidative stress in different organs and species of living organisms by the generation of free radicals which in turn can lead to protein and lipid peroxidation, DNA damage, and apoptosis, and likewise decrease the antioxidant enzymes (SOD, CAT, and GPX) activities (Kalender et al., 2012; Saulsbury et al., 2009; Verma et al., 2007). In this study, CPF confirms to induce an increase in the levels of TBARS and a decrease in the levels of GSH, and the activities of the antioxidant enzymes (GPx, CAT, SOD, and GST) in plasma and testis (Table 4) at dose level with clear general toxicity.

The present results showed that treatment with CPF caused significant decline in semen quality (Table 2). Meeker et al. (2006), Piña-Guzmán et al. (2006), El Mazoudy et al. (2011), and Shittu et al. (2012) have found that OP compounds alter male reproductive function, particularly semen quality. Epidemiological study showed that in Chinese pesticide factory workers, OP exposure was associated with a decrease in sperm concentration, motility, and testosterone level and an increase inLH and higher sex chromosome aneuploidy in semen (Perry et al., 2007).

OP pesticides such as parathion, methyl parathion, dimethoate, and CPF are endocrine disrupters. In fact, they are structurally similar to various sexual hormones and may interact with hormone receptors, hormone action, and metabolism by activation or inhibition of the enzyme activities that involved in steroid hormones synthesis and/or with induction of gene expression. This might alter the endogenous hormones levels and result in a deflection from normal male developmental programming and reproductive

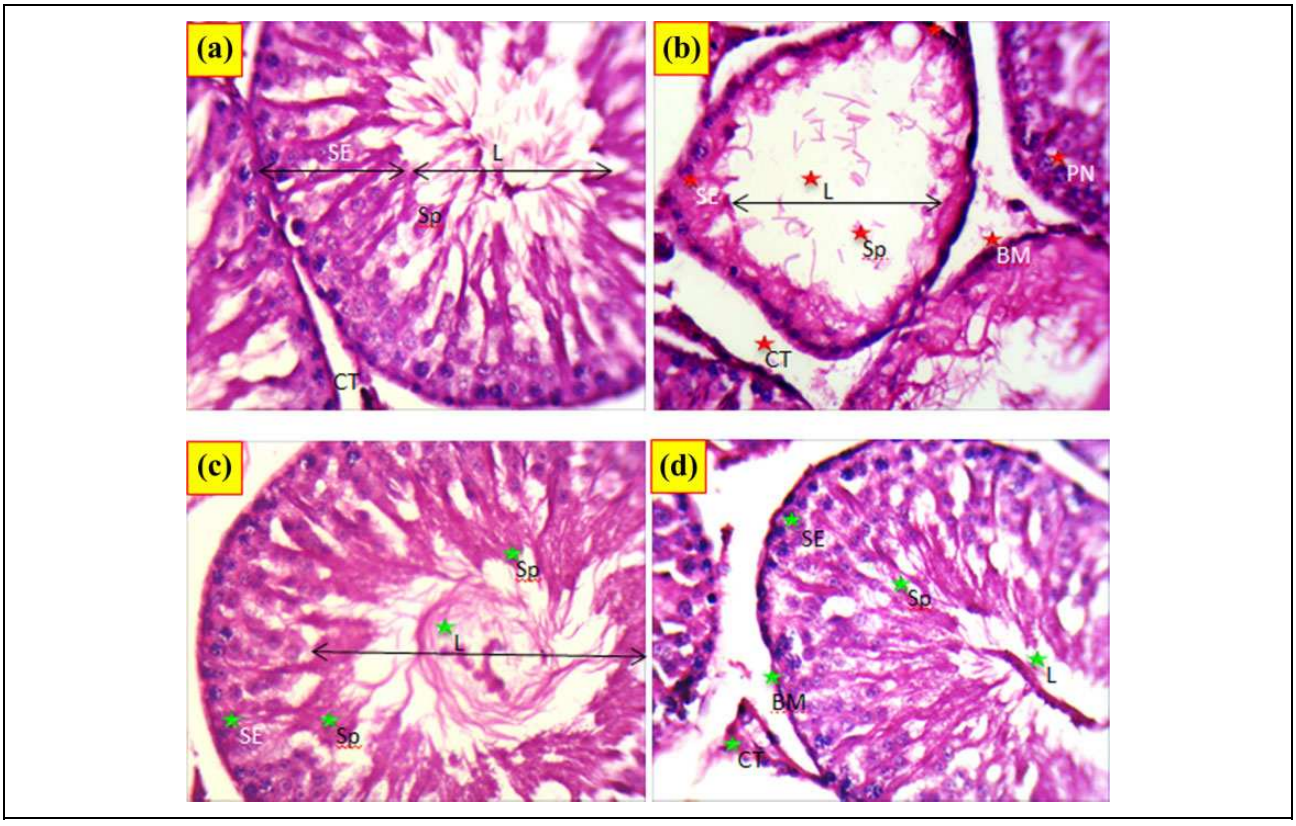

Figure 2. Light microscopic sections of testes from various examined groups: control (a), CPF (b), NSO (c), and coadministration NSO + CPF (d) for 4 weeks. Sections (5 μm) were stained with hematoxylin–eosin (×400). L: lumen; CT: connective tissue; SE: seminal epithelium; Sp: sperm; BM: basal membrane; PN: picnotic nucleus; V: vacuolation; red star ★: deleterious effect; green star ★: ameliorating effect; CPF: chlorpyrifos; NSO: *Nigella sativa* oil.

tract growth and function (De Angelis 2009; Luccio-Camelo and Prins, 2011; Tait et al. 2009).

As a possible mechanism for the antigonadal action of OPs, they may change the concentration of neurotransmitters, affecting the pituitary gland causing changes in gonadotropin concentrations and thus subsequent spermatogonic impairment (Sarkar et al., 2000; Tait et al. 2009).

In the current study, the detrimental effects of CPF on reproductive performance can be attributed to: (1) its effects on hormonal balance of hypothalamic–pituitary–gonadal and/or –thyroid axis (FSH, LH, testosterone, T4) that are involved in the control of steroidogenesis, spermatogenesis, and spermiation as shown in Table 3, (2) its capacity to enhance the production of ROS that causes lipid peroxidation in spermatozoa membrane, sperm DNA damage and to inhibit the activities of antioxidant enzymes (Table 4), (3) to decrease the expression of testicular steroidogenic acute regulatory (StAR) protein and in steroidogenic (3β-HSD and 17β-HSD) enzymes, which help in the transfer of cholesterol in mitochondria and testosterone biosynthesis (Manna et al., 2001). Indeed, the present results showed that the activity of 17-KSR that convert androstenedione to testosterone was significantly decreased in rats treated with CPF compared with the control group (Table 3).

The significant decline in the absolute and relative reproductive organ weights (testes, epididymides, and seminal vesicles; Table 1), sperm and spermatid count, motility (Table 2), and testosterone level (Table 3), as well an elevation in dead and abnormal sperm rates (Table 2) are accompanied by histopathological changes in testis that pronounced by alterations of spermatogenesis with a complete loss of all the stages of germ cell maturation, Sertoli cell toxicity, mild to severe degenerative aspects of seminiferous tubules, and widening of interstitial spaces (Figures 1 and 2).

As shown in Table 3, hormone analysis showed that CPF caused significant reduction in testosterone and FT4 and induction of LH and FSH levels as compared to control. These may be responsible for impaired male reproductive system suggesting the

presence of CPF in rats. Rats subjected to prolonged thyroid hormone deficiency showed marked morphological and functional testicular alterations. Thyroid hormone was shown to play a critical role in maintaining steroidogenesis, spermatogenesis, and metabolic processes in testis (Wagner et al., 2008) and increase the expression of StAR protein in the Leydig cells (Manna et al., 2001). Consistent with our results (Tables 2 and 3), it was reported that CPF induced several histopathological changes in the testes (Kalender et al., 2012), a depression of sperm parameters and plasma T4 level (De Angelis et al., 2009; Rawlings et al., 1998), gene expression of gonadotropin-releasing hormone in hypothalamic cell line GTI-7 (Gore, 2001), and may interfere with testosterone metabolism, potentially leading to hormonal unbalance and poor SC (Meeker et al., 2006). In addition, antiandrogenic activity for CPF has been described *in vitro* by using NIH3T3 cell line stably expressing human androgen receptor (Viswanath et al., 2010). Mariana et al. (2009) found that dimethoate (OP), glyphosate, and zineb (carbamate), either alone or in combination induced increase in the oxidative damage in the plasma, liver, and testes, leading to a decrease in testosterone and an increase in the levels of FSH and LH in the treated rats, and this is a coincidence with the present results (Table 3). Therefore, oxidative stress may be partly responsible for the vital and reproductive organs injury and dysfunction.

N. sativa *oil*

Our data (Table 1) showed that treatment with NSO alone caused significant increase in the relative weights of the reproductive organs. The coadministration of NSO with CPF reversed markedly the adverse effects of CPF within control levels, and this may be due to the antioxidant activities of bioactive components of *N. sativa* (Table 4). Recent studies were performed on the antioxidant activity of essential oil of *N. sativa* and its effects on antioxidant enzyme status; it was found that TQ, carvacrol, 4-terpineol, anethol, dithymoquinone, and thymol have proper radical scavenging properties. It appears also that TQ (the major active constituent of seed oil extract) in turn exhibits cerebral, renal, liver, and cardiac protective effect against many xenobiotics through its antioxidant action and ability to boost antioxidant enzymes activities in animals (Mohamadin et al., 2010).

The results obtained in the present work indicated that treatment with NSO normalized all altered semen parameters to the normal status levels in rats treated with CPF; again, this protective effect might be due to its antioxidant capacity (Table 4). Also NSO alone improved semen charactristics (Table 2). As a support to this data, *N. sativa* is known for its action in ameliorating reproductive performance in male rats (Al-Sa'aidi et al., 2009). Moreover, both the crude fixed oil of *N. sativa* and TQ has been found to inhibit membrane lipid peroxidation (Kanter, 2011) and to enhance the antioxidant defense systems (Mohamadin et al., 2010).

Treatment with NSO significantly increased the activities of 17-KSR and decreased 17β-HSD compared with the control. Indeed, NSO increased the process of steroidogenesis and hence testosterone production (Table 3), improving sperm proliferation (Table 2). Also the presence of NSO with CPF caused significant increase in the decline of 17-KSR compared with the group of CPF. The decline in the activity of 17-KSR in animals treated with CPF indicates its adverse effects on the steroidogenic process for production of testosterone which in turn affects the process of fertility via decreasing testosterone hormone production (Table 3) and sperm production, and proliferation and hence motility, count, and abnormality (Table 2).

Recent subchronic studies were performed on the possible ameliorating role of the ethanolic and aqueous extracts of *N. sativa* seeds or their fixed oil on male fertility; the results revealed a significant increase in the weight of reproductive organs, sperm motility, spermatids, and SC as well as decreased lipid peroxidation level and improved activities of antioxidant enzymes GPx, SOD, and CAT. Moreover, overall reproductive parameters showed increased testosterone and FSH levels and decreased excitation time of first mount, first ejaculation, and second trail (Al-Sa'aidi et al., 2009). El-Tohamy et al. (2010) found that *N. sativa* has the best promising role in improving the semen parameters and immunity as well as reducing free radicals generation more than *Raphanus sativus* and *Eruca sativa*.

The administration of NSO alone significantly increased plasma testosterone, LH, and FSH levels. Furthermore, the coadministration of NSO with CPF showed a marked adjustment in hormone level imbalance induced by CPF and subsequently improved semen quality (Tables 2 and 3). Regarding these findings, El Khasmi et al. (2011) found that treatment with *N. sativa* showed a significant increase in the weight of reproductive organs and circulating levels

of testosterone, FSH, and LH in rats. This may result from the activation of hypothalamic–pituitary–testicular axis, which stimulates steroidogenesis and spermatogenesis processes (El Khasmi et al., 2011).

The histopathological examinations of testes showed that NSO coadministration alleviated evidently adverse effects induced by CPF. In accordance with our results, the histological investigation of testis and epididymis in rats treated by aqueous or alcoholic extract of *N. sativa* seeds showed an increase in the spermatogenesis activity, seminiferous tubules thickness, and diameters as well as in the diameter of Leydig cells, sperm density in the lumen of seminiferous tubules, and epididymis ducts, which in turn presented higher epithelial cells (Al-Sa'aidi et al., 2009). Our data supported those obtained by Wahba (2011) who reported that pretreatment of male rats with *N. sativa*, linseed, and celery oils for 4 weeks produced a protective effect against testicular injury induced by sodium valproate. This effect was manifested by increased weight of the testis, improved semen quality and quantity, elevated serum testosterone level, decreased lipid peroxidation in the testis as well as alleviation of degenerative changes in testes of rats given sodium valproate. The mechanism for this protective effect of NSO against the toxic effects of CPF is due its free radical scavenging activity and increased antioxidant enzymes in rats.

Conclusion

This study indicated that coadministration of NSO and CPF reverses changes partly or completely in the relative reproductive, vital organs weights, semen characteristics, hormone levels, oxidative damage, antioxidant enzymes, and the histopathological injuries of testes within the normal status and thereby improved semen quality. Also, NSO can improve semen picture and moderate CPF-induced reproductive toxicity by its antioxidant properties.

Highlights

- OP compounds such as CPF induced reproductive toxicity.
- Oxidative stress is the main mechanism of adverse effects.
- The oxidative stress is known to be a key factor in several diseases.
- Herbalism is an alternative or folk medicine for medicinal purposes.
- NGO moderates CPF-induced reproductive toxicity.

Conflict of interest

The authors declared no conflicts of interest.

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