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Protective Effects of *Nigella sativa* extract against Chromiumvi-Induced Genotoxicity in Nile Tilapia (*Oreochromis niloticus*) and Zebrafish (*Danio rerio*)

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Abstract: Chromium is a matter of an increasing health concern for aquatic environments and Cr(VI)is the dominant toxicant at some sites in Egypt. The aim of this study was to evaluate the genotoxicty potential of Cr(VI)in Nile tilapia and zebra fish using RT-PCR and possible protective effects of *N. sativa*. Fish from Nile tilapia species were divided into 8 groups and treated with Cr(VI) alone or in combination with the crude extract of *N. sativa*, *N. sativa* oil, or its derivative thymoquinone. The semi-quantitative RT-PCR results indicated that treatment with Cr(VI) at 4.37and 1.75 mg/L for tilapia and zebra fish, respectively resulted in a significant increase in hepatic and brain mRNA level of cytochrome including CYP1A2, CYP3A and CYP2E1 in both fish species as compared to control group. Moreover, Cr(VI) was found to induce severe histological changes in all organs of the tested fish. On the other hand, the combined treatment showed that mRNA level of genes decreased significantly in the groups treated with Cr(VI) plus *N. sativa* oil or thymoquinone as compared to the groups treated with the crude extract or Cr(VI) alone accompanied with a significant improvement in the histological picture. Moreover, *N. sativa* essential oil was found to be more effective against Cr(VI) hazards and may be a promise candidate against different environmental pollutants.

Key words: Chromium · Essential oil · *Negilla sativa* · Nile Tilapia · Thymoquinone

INTRODUCTION

Exposure to chromium is an increasing concern for the health of the marine environment. Chromium enters the marine environment primarily via surface water runoff from rivers and deposition from the atmosphere. Contamination of the environment by heavy metals has been increasing every year [1,2] and the analysis of the cytotoxic effects of such metals has received special attention due to the fact that they are potentially mutagenic and induce the formation of tumors in experimental animals and humans [3].

Chromium compounds are known to have toxic, genotoxic, mutagenic and carcinogenic effects on man and animals [4]. Trivalent chromium (CrIII) and hexavalent chromium (CrVI) are being biologically active but differing in their ability to cross biological membranes.

Several *in vivo* and *in vitro* studies have shown that chromium compounds damage DNA in a variety of ways, including DNA single and double-strand breaks (SDSBs) generating chromosomal aberrations, micronucleus formation, sister chromatid exchanges, formation of DNA adducts and alteration in DNA replication and transcription [5,6].

Black seed (*Nigella sativa* L.), has been employed for thousands of years as a spice and food preservative, as well as a protective and curative remedy for numerous disorders [7]. Several beneficial pharmacological effects

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have been attributed to various crude and purified components of black seed, including antihistaminergic, antihypertensive, hypoglycemic, antimicrobial, mast cell stabilizing, antioxidant and antiinflammatory activities [8,9]. Black seed preparations have also demonstrated significant *in vitro* and *in vivo* antineoplastic activity [10].

Fish are used as a test organism in which it is possible to detect DNA damage induced by direct mutagens and pro-mutagens in both fresh and salt water [11,12]. Thus, genotoxic pollutants may lead to the contamination not only of the aquatic organisms themselves but of the entire ecosystem and, finally, of humans through the food chain. Therefore, the main objectives in the present study were twofold: (i) to evaluate the genotoxicty potential of chromium VI(CrVI) in Nile tilapia (*Oreochromis niloticus*) and zebrafish (*Danio rerio*) in order to evaluate the possible role of cytochrome P450 in its metabolism to more potent mutagenic products using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) and (ii) to evaluate the anticlastogenic activity of prolonged supplementation with the crude extract of *N. sativa,* N. sativa oil or its derivative thymoquinone in Nile tilapia and zebrafish treated with Cr(VI).

MATERIALS AND METHODS

Reagents: Reagents for RFLP-PCR method were purchased from Invitrogen (USA). CrVI were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents and chemicals were of the highest purity commercial available.

Preparation of *Nigella Sativa* Crude Extract, Oil and Thymoquinone

Preparation and Analysis of *N. Sativa* Essential Oil: Black seed essential oil was prepared according to the procedure described previously [13]. Seventy five grams of black seed were crushed and extraction was applied using about 220 ml of light petroleum ether (b.p. =40, 60°C) in a Soxhlet apparatus. Extraction of the aqueous distillate was carried out with n-hexane and the solvent was removed from the extract under vacuum to yield the essential oil. The thymoquinone was isolated and determined by HPLC according to the procedure reported by Ghosheh *et al.* [14].The column was an Alpha Bond C_{18} type,10 l 300 3.9 mm. The isocratic mobile phase consisted of H_2O: methanol: 2-propanol in the ratio of 10:9:1 by volume. Column temperature and the flow rate were 28°C and 1 ml/min, respectively. The detector was a DAD (254.4 nm) and the injection volume was 5 µl.

Preparation of Ethyl-Acetate and Butanol Extracts: One kilogram *N. sativa* L. seeds was washed, dried and crushed to a powder with an electric micronizer. The powder was exhaustively extracted with 90% ethanol at room temperature and the extract was centrifuged at 10,000 *g* for 15 min to remove residual solid debris. The clear supernatant was then concentrated under reduced pressure. The concentrated extract was partitioned between 10% methanol-water and n-hexane. After removing the n-hexane fraction, the aqueous layer was partitioned again with ethyl acetate, followed by n-butanol. The yields of the butanol and ethyl acetate extracts were 2.1 and 0.23% (w/w), respectively. Analysis by nuclear magnetic resonance showed that the butanol extract contained two saponosides derived from a-amyrin. However, the ethyl acetate extract did not contain polyphenols and did contain monoxideterpenes and it was discarded.

Fish: *Tilapia zillii* fish (52.6 ± 5.7 g) and zebrafish (0.49±0.01 g) were purchased from El-Wafaa fish farm, Giza, Egypt and were transported in large plastic water containers supplied with battery aerators as a source of oxygen. Fish were maintained on *ad libitum* standard fish food at the Genetic of Hydrobiology Lab. National Research Centre (Dokki, Cairo, Egypt). After an acclimation period of 1 week, tilapia and zebrafish were divided separately into eight experimental groups (10 fish/ group) and were placed into fish aquariums containing de-chlorinated tap water (26.7±2.1°C and pH 7.2-8.2).

Experimental Design: Tilapia and zebrafish within different treatment groups (Table 1) were treated orally for 30 consecutive days as follows: group 1, untreated control; group 2, treated with CrVI (4.37 mg/kg b.w for tilapia or 1.75 mg/ kg b.w for zebrafish, i.e. 1/10 LD_{50}); group 3, fed fish diet supplemented with 0.5 mg/kg *N. sativa* oil; group 4, fed fish diet supplemented with 0.5 mg/kg thymoquinone; group 5, fed fish diet supplemented with 0.5 mg/kg crude extract of *N. sativa*; groups 6, 7 and 8 fed fish diet supplemented with the three protective agents and treated with Cr(VI) at the same doses. At the end of the experimental period, all fish were sacrificed and dissected on day 31. Five liver and brain samples from tilapia as well as five liver and the whole head samples from zebrafish within different treatment groups were

Table 1: Experimental groups and the respected treatments (n = 10)*

Groups	Treatment	Dose (mg/ kg b.w.) Tilapia zillii	Zebrafish
1	Untreated control	---	---
2	CrVI	4.37 mg/ kg b.w	1.75 mg/ kg b.w
3	*N. sativa* oil	0.5 mg/kg diet	0.5 mg/kg diet
4	thymoquinone	0.5 mg/kg diet	0.5 mg/kg diet
5	*N. sativa* crude extract	0.5 mg/kg diet	0.5 mg/kg diet
6	*N. sativa* oil + Cr(VI)	4.37mg/kg b.w + 0.5 mg/kg diet	1.75 mg/kg b.w + 0.5 mg/kg diet
7	thymoquinone + Cr(VI)	4.37mg/kg b.w + 0.5 mg/kg diet	1.75 mg/kg b.w + 0.5mg/kg diet
8	*N. sativa* crude extract + Cr(VI)	4.37mg/kg b.w + 0.5 mg/kg diet	1.75 mg/kg b.w + 0.5 mg/kg diet

* *N. sativa* oil, *N. sativa* crude extract and thymoquinone were mixed with the diet whereas; Cr(VI) was dissolved in distilled water

Table 2: Primers and reaction parameters in RT-PCR

Target cDNA	Primer name Primer sequence (5´–3´)	PCR product size (bp)
β-Actin	Act-F CCCCATCGAGCACGGTATTG	189
	Act-R ATGGCGGGGGTGTTGAAGGTC	
CYP1A2	CYP1A2-F AAGATCCATGAGGAGCTGGA	139
	CYP1A2-R TCCCCAATGCACCGGCGCTTTCC	
CYP3A	CYP3A-F GAAGCATTGAGGAGGATCAC	376
	CYP3A-R GGGTTGTTGAGGGAATCCAC	
CYP2E1	CYP2E1-F GCGGTTCTTGGCATCACCGT	447
	CYP2E1-R GCAGGGTGCACAGCCAATCA	

collected in liquid nitrogen and stored at -80°C until use for RNA extraction. The other five fish within all groups were collected for histopathological assay according to the methods described by Roberts [15].

Molecular Analysis

Semi-Quantitative RT-PCR

Rna Extraction: Total RNA was isolated from 50 to 100 ìg of liver or brain tissue by the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 ìl molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase and removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol.

Reverse Transcription: The complete Poly (A) + RNA isolated fish samples were reverse transcribed into cDNA in a total volume of 20 µl using 1 µl oligo(dT) primer. The composition of the reaction mixture, termed as master mix (MM), consisted of 50 mM $MgCl_2$, 10x reverse transcription (RT) buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3 Perkin-Elmer), 10 mM of each dNTP (Amersham, Brunswick, Germany) and 50 µM of oligo(dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through polymerase chain reaction (PCR).

Polymerase Chain Reaction (PCR): The first strand cDNA of different fish samples was used as templates for RT-PCR with the specific primers of cytochrom 450 genes. The sequences of specific primer and product sizes are listed in Table (2). β-Actin was used as a housekeeping gene for normalizing mRNA levels of the target genes. The reaction mixture for RT-PCR was consisted of 10 mM dNTP's, 50 mM $MgCl_2$, 10x PCR buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.3, Gibco BRL, Eggenstein, Germany) and autoclaved water. RT-PCR amplification with CYP1A2, 3A and 2E1 gene-specific primers was performed for 35, 25 and 19 cycles, respectively. The PCR cycling parameters were one cycle of 94°C for 3 min, 35, 25 or 19 cycles of 94°C for 40 s, 54°C for 40 s, 72°C for 40 s and a final cycle of 72 °C for 7 min. The PCR products were then loaded onto 2.0% agarose gel, with PCR products derived from β-actin of the different fish samples. Each experiment was repeated with five fish, generating new cDNAs at least five times and repeating each PCR reaction at least five

Statistical Analysis: The binomial data for semi-quantitative RT-PCR analysis showed normal distribution. Therefore, all data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [16] followed by Scheffé-test to assess significant differences between groups. All statements of significant were based on probability of $P < 0.05$.

RESULTS

Semi-Quantitative RT-PCR: Expression of cytochrome p450 gene family in liver and brain of tilapia as well as zebrafish is summarized in Figures 1, 2 and 3. It is clear that gene expression level of all cytochrome p450 genes studied was higher in liver than brain tissues. In addition, the results revealed a significant ($P \leq 0.05$) increase in hepatic and brain mRNA level of cytochrome p450 gene family including CYP1A2, CYP3A and CYP2E1 in both fish species treated with Cr(VI) compared to the control and the other treated groups. On the other hand, treatment of tilapia and zebrafish with *N. sativa* oil, thymoquinone or the crude extract of *N. sativa* did not cause any

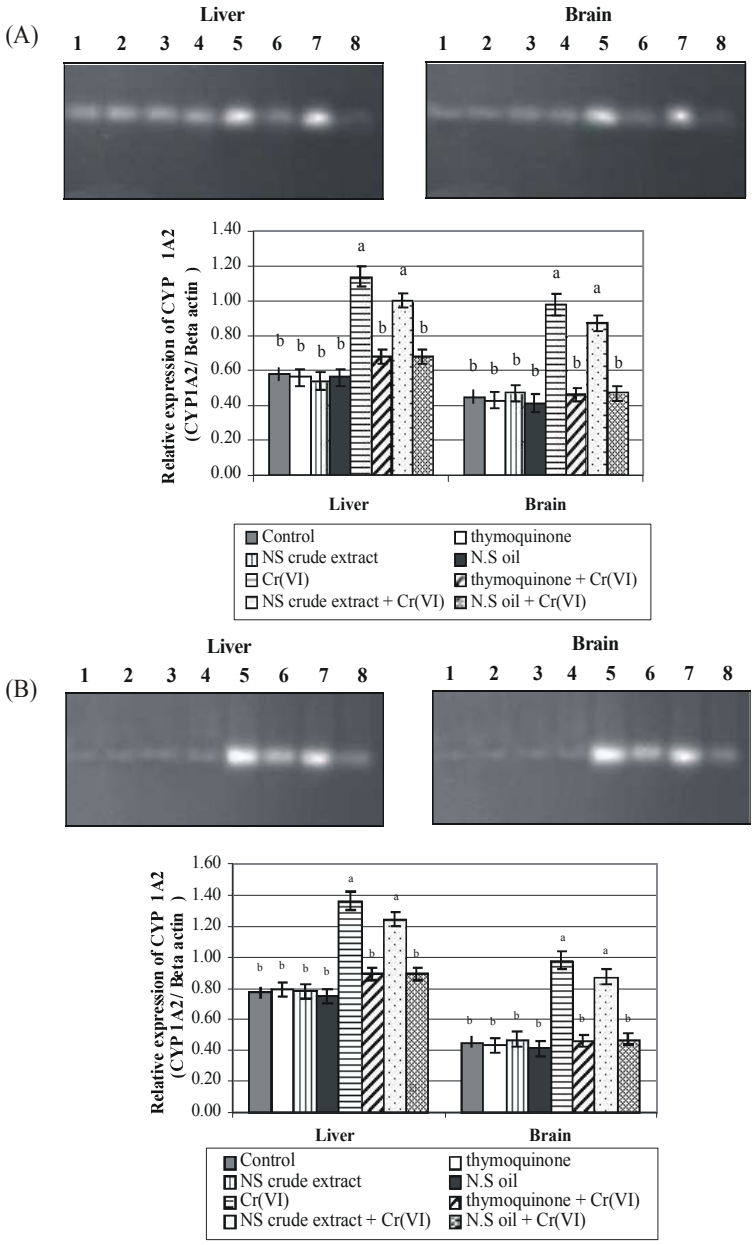

Fig. 1: CYP1A2 mRNA expression in the liver and brain of *Tilapia zillii* (A) and zibrafish (B) determined by semi-quantitative RT-PCR. A and B agarose-gel electrophoresis of RT-PCR products for control (lane 1), thymoquinone (lane 2), *N. sativa* crude extract (lane 3), *N. sativa* oil (lane 4), Cr (lane 5), thymoquinone + Cr (lane 6), *N. sativa* crude extract + Cr (lane 7) and *N. sativa* oil + Cr (lane 8). Within each column, means superscripts with different letters are significantly different ($P \leq 0.05$).

mutagenicity in the liver or brain tissues. Where, the expression of CYP1A2, CYP3A and CYP2E1 in both fish species treated with the three agents was down regulated.

The results of the antimutagenic activity in groups fed diet supplemented with *N. sativa* oil, its derivative thymoquinone or the crude extract in the diet against Cr(VI) treatment in Nile tilapia and zebrafish indicated that *N. sativa* oil and thymoquinone were able to inhibit the mutagenicity of Cr(VI) in both fish species. The mRNA level of CYP1A2, CYP3A and CYP2E1 was significantly lower in the groups treated with Cr(VI) plus *N. sativa* oil or thymoquinone than in the group treated with Cr(VI)

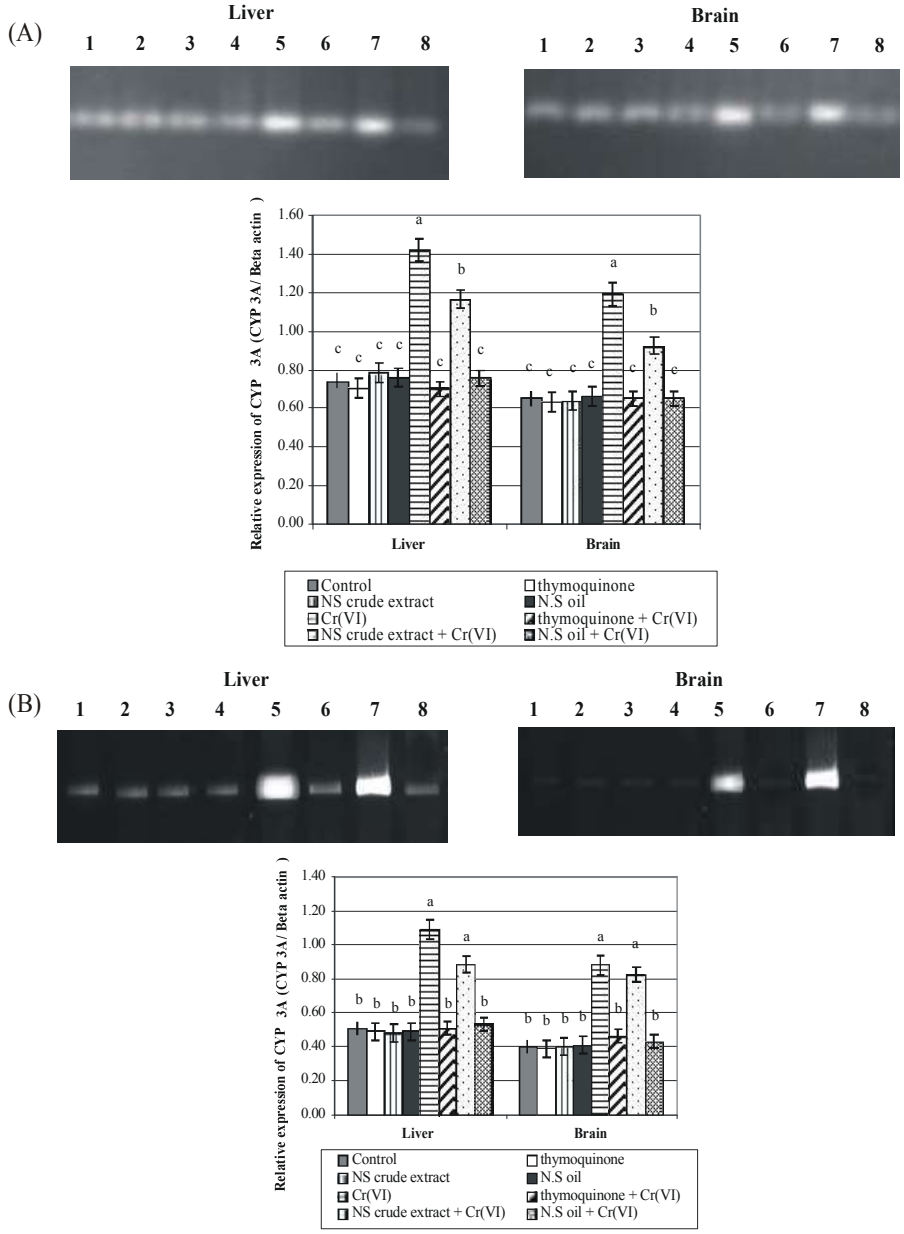

Fig. 2: CYP3A mRNA expression in the liver and brain of *Tilapia zillii* (A) and zibrafish (B) determined by semi-quantitative RT-PCR. A and B agarose-gel electrophoresis of RT-PCR products for control (lane 1), thymoquinone (lane 2), *N. sativa* crude extract (lane 3), *N. sativa* oil (lane 4), Cr (lane 5), thymoquinone + Cr (lane 6), *N. sativa* crude extract + Cr (lane 7) and *N. sativa* oil + Cr (lane 8). Within each column, means superscripts with different letters are significantly different ($P \leq 0.05$).

alone. However, the crude extract of *N. sativa* was not able to inhibit the mutagenic activity of Cr(VI). Where, the mRNA level of CYP1A2 gene in the liver and brain tissues of both species in this group was not significantly different than in the group treated with Cr(VI) alone. Despite the mRNA level of CYP3A gene in crude extract of *N. sativa* plus Cr(VI) group was significantly different than in the group treated with Cr(VI) alone in the liver and brain of tilapia; it was still significantly higher than in the control and *N. sativa* oil or thymoquinone-treated groups.

The same trend was observed for CYP2E1 gene. The mRNA level of CYP2E1 gene in the liver or brain of tilapia and zebrafish in the same group was significantly different than in the group treated with Cr(VI) alone but its

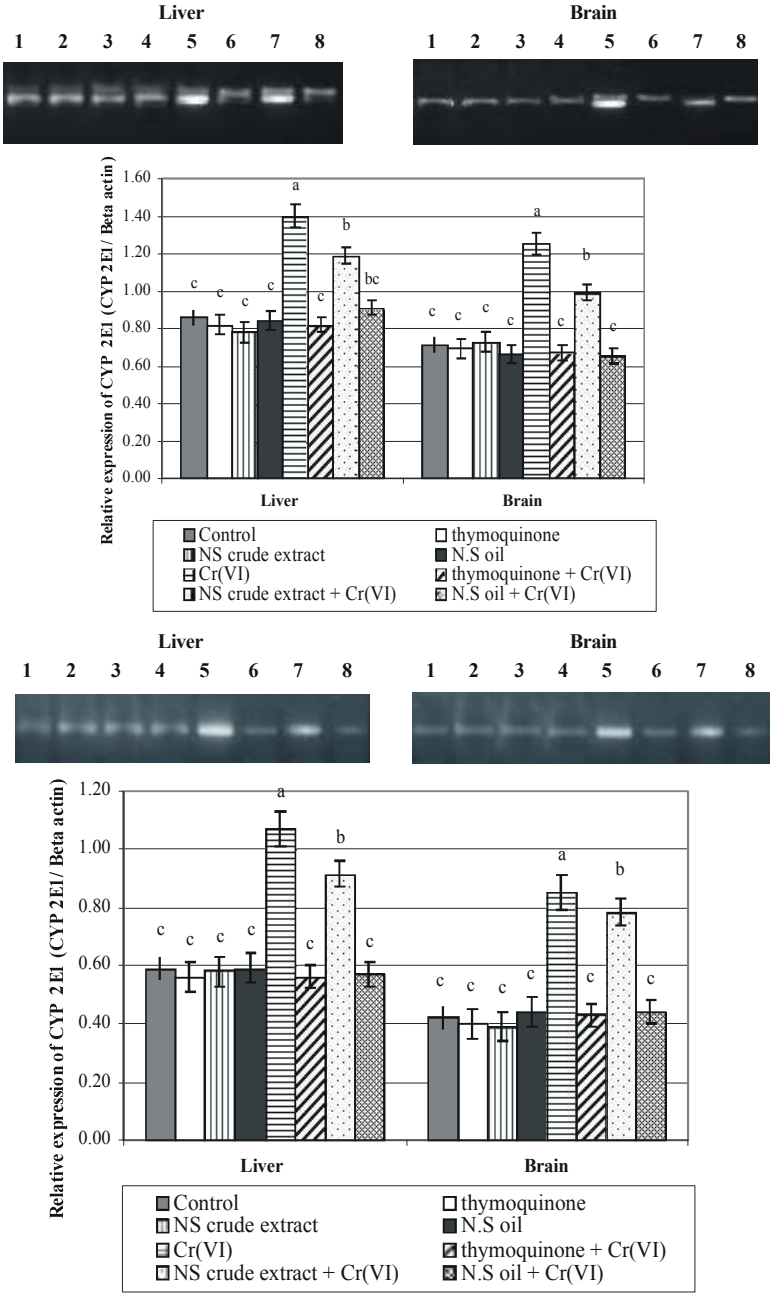

Fig. 3: CYP2E1 mRNA expression in the liver and brain of *Tilapia zillii* (A) and zibrafish (B) determined by semi-quantitative RT-PCR. A and B agarose-gel electrophoresis of RT-PCR products for control (lane 1), thymoquinone (lane 2), *N. sativa* crude extract (lane 3), *N. sativa* oil (lane 4), Cr (lane 5), thymoquinone + Cr (lane 6), *N. sativa* crude extract + Cr (lane 7) and *N. sativa* oil + Cr (lane 8). Within each column, means superscripts with different letters are significantly different ($P \leq 0.05$).

level was still significantly higher than in the control, *N. sativa* oil or thymoquinone-treated groups. On the other hand, there was no significant change in the levels of β-Actin gene in all groups throughout the study.

The histological examination of zebrafish treated with Cr(VI) alone showed degenerative changes and necrosis of the liver with accumulation of fat in the hepatic cells (Fig. 4a). Zebrafish treated with Cr(VI) plus *N. sativa* oil, its derivative thymoquinone or the crude extract showed

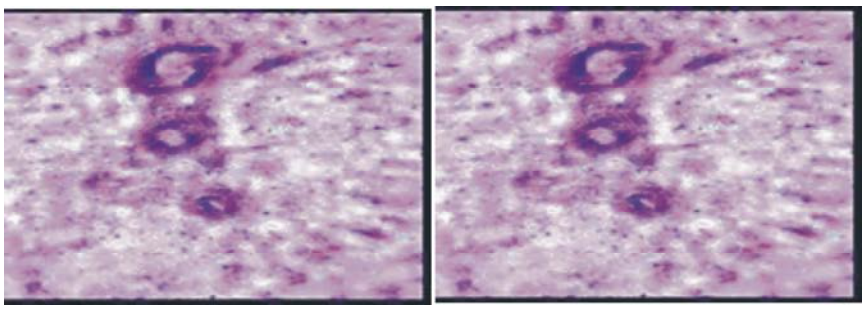

Fig. 4: Microscopic examination of liver section of zebrafish showing: (a) fatty degeneration in the hepatocytes in the Cr alone-treated group, (b) slight degenerative changes in hepatocytes in the groups treated with Cr plus *N. sativa* oil, thymoquinone or the crude extract. (H and E X100)

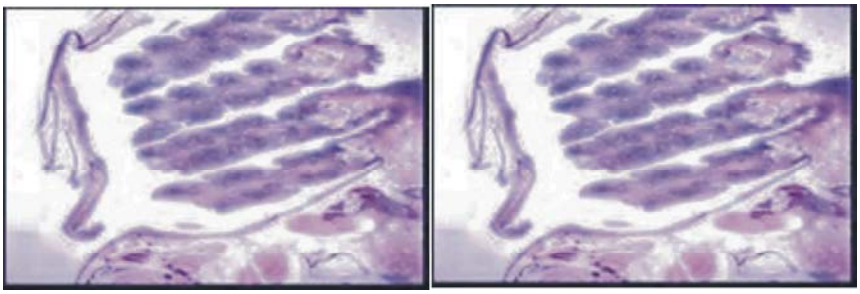

Fig. 5: Microscopic examination of the gills section of zebrafish showing: (a) severe hyperplasia in the group treated with Cr alone, (b) hyperplastic with fusion in between the secondary lamellae in the group treated with Cr plus *N. sativa* oil, thymoquinone or the crude extract.(H and E X100)

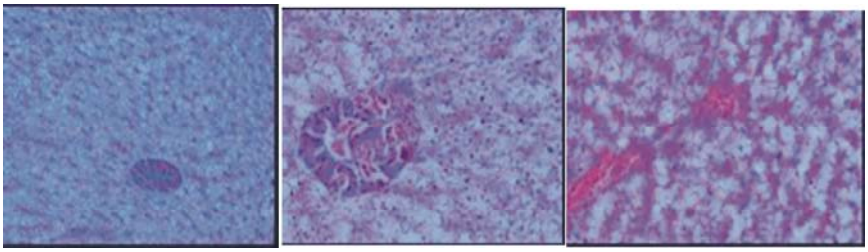

Fig. 6: Microscopic examination of liver of *Tilapia zillii* fish showing: (a) congetion and vacuolar degeneration in fish treated with Cr alone, (b) degenerative changes in both hepatocytes and pancreatic tissues associated with inflammatory cells infiltration in Cr alone-treated group, (c) slight normal hepatocytes and pancreatic tissues in the groups treated with Cr plus *N. sativa* oil, thymoquinone or the crude extract.(H and E X100)

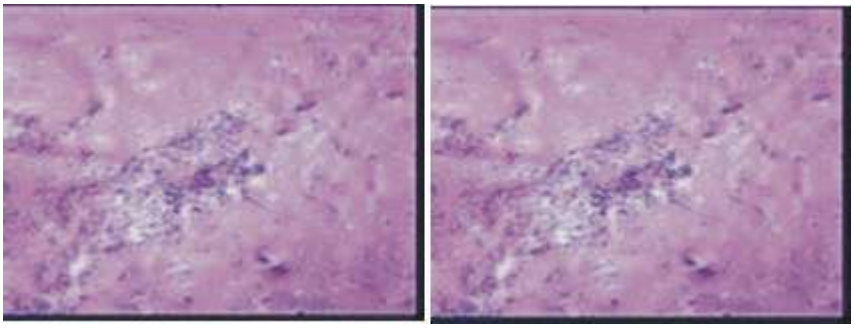

Fig. 7: Sections of brain of *Tilapia zillii* fish showing: (a) focal area of gliosis in Cr alone treated group, (b) depletion in the granular cell layer in the fish treated with Cr plus *N. sativa* oil, thymoquinone or the crude extract. (H and E X 100)

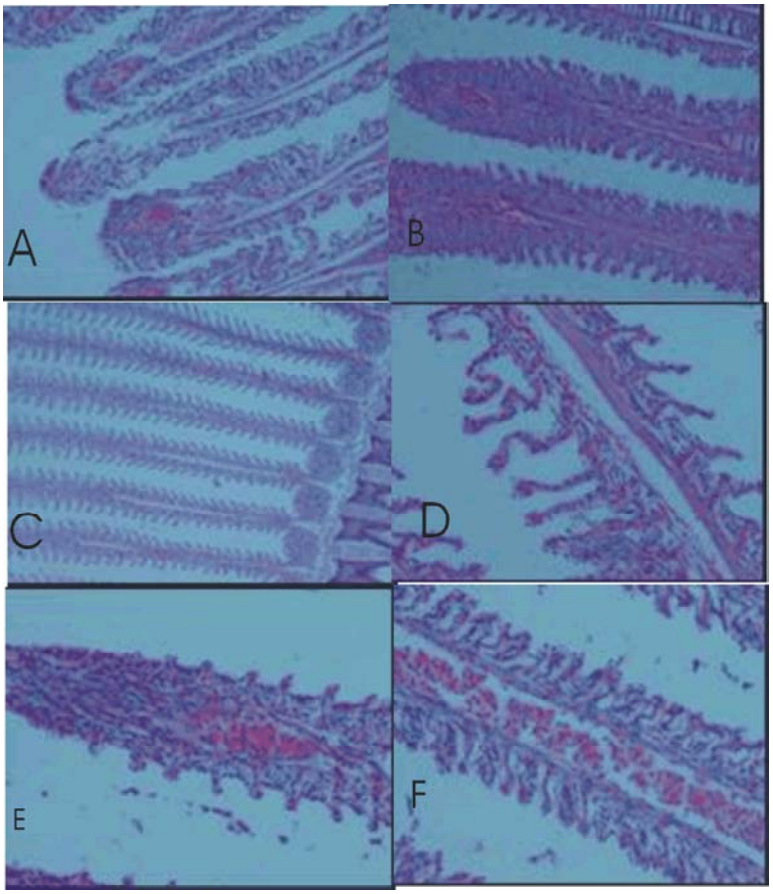

Fig. 8: Sections of gills of *Tilapia zillii* fish showing: (a and b) congetion and hyperplasia in the secondary lamellae associated with degenerative changes and necrosis in the epithelial lining the secondary lamellae in Cr alone-treated group, (c) normal lamellae in the group treated with *N. sativa* oil alone, (d) slightly hyperplasia at the base of the secondary lamellae in the group treated with the crude extract of *N. sativa* alone, (e) congetion and hyperplasia in the secondary lamellae associated with degenerative changes and necrosis in the epithelial lining the secondary lamellae in the group treated with Cr plus *N. sativa* oil or thymoquinone, (f) congetion and hyperplasia in the secondary lamellae associated with degenerative changes and necrosis in the epithelial lining the secondary lamellae in the group treated with Cr plus the crude extract of *N. sativa*. (H and E X 100)

slight degenerative in the liver tissue although; *N. sativa* oil was more effective in the protection against Cr(VI) toxicity (Fig. 4b). The gills of zebrafish treated with Cr(VI) alone showed severe hyperplasia (Fig. 5a) whereas; gills of zebrafish received the combined treatments with Cr(VI) plus the three protectives showed moderate hyperplastic with fusion in between the secondary lamellae (Fig. 5b).

The histological examination of the liver of Tilapia fish in Cr(VI)-treated group revealed congestion and vacuolar degeneration (Fig. 6a) and degenerative changes in both hepatocytes and pancreatic tissues associated with inflammatory cells infiltration (Fig. 6b). Liver of Tilapia fish treated with Cr(VI)plus *N. sativa* oil, its derivative thymoquinone or the crude extract showed slightly normal hepatocytes and pancreatic tissues (Fig. 6c). The brain of Tilapia fish in the control group or those treated with *N. sativa* oil, thymoquinone or the crude extract alone or in combination with Cr(VI) showed normal histological picture. However, the brain of Tilapia treated with Cr(VI) alone showed focal area of gliosis (Fig. 7a) and depletion in the granular cell layes (Fig. 7b).

Microscopic examination of the sections of gills of Tilapia fish treated with Cr(VI) alone showed congestion and hyperplasia in the secondary lamellae associated with degenerative changes and necrosis in the epithelial lining the secondary lamellae (Fig. 8a,b). Gills of the groups treated with *N. sativa* oil showed slightly normal lamellae (Fig. 8c) whereas, those in the group treated with either

the crude extract or thymoquinone showed slightly hyperplasia at the base of the secondary lamellae (Fig. 8d). Gills of the groups received the combined treatments of Cr(VI) plus the protective agents showed moderate congestion and hyperplasis in the secondary lamellae associated with moderate degenerative changes (Fig. 8e) and necrosis in the epithelial lining the secondary lamellae (Fig. 8f)

DISCUSSION

Chromium is stable both as Cr(VI) and Cr(III), although few environmental studies distinguish between the two forms. The more toxic hexavalent form is more prevalent in the marine environment [17]. In the present study, the mutagenicity of Cr(VI) was evaluated in *Oreochromis niloticus* and *Danio rerio* using the semi-quantitative RT-PCR and the protective role of *N. sativa* and its components against Cr(VI)-induced genotoxicity. The selected doses of *N. sativa* and its components were based on the work of Abdel-Wahhab and Aly [9] however; the doses of Cr (VI) were based on the work of Domingues *et al.* [18] for zebra fish and the work of Prabakaran *et al.* [19] for tilapia. The results revealed a significant increase in hepatic and brain mRNA level of cytochrome p450 gene family including CYP1A2, CYP3A and CYP2E1 in both fish species treated with Cr(VI) than control and other treated groups. Similar to the current observations, Calamari *et al.* [20] reported that the genotoxic effect of chromium was more pronounced in the liver than other organs in adult males Salmo gairdneri fish exposed to chromium. In addition, Lehman-McKeeman *et al.* [21] found that exposure of mice to the environmental pollution increased liver weight by about 65% and increased microsomal cytochrome P450 content 2-fold over the control level, which were consistent with increasing of CYP2B, CYP1A1, 1A2 and 3A mRNA and protein levels as well as induction of liver tumors in mice. Therefore, the effect of environmental pollution on cytochrome p450 gene induction is pretranslational and most likely occurs by transcriptional activation of this family gene.

The biological mechanism of Cr(VI) to induce mutagenicity is not clearly understood. In addition, Salnikow and Zhitkovich [22] reported that chronic exposure to Cr(VI) has long been known to increase cancer incidence among affected individuals as a result of DNA damage. Cr(VI) can also cause significant changes in DNA methylation and histone modifications, leading to reactivation of gene expression. Therefore, it could be suggested that the mutagenicity of Cr(VI) in tilapia and zebrafish is attributed to transcriptional reactivation of cytochrome p450 family genes.

The results of antimutagenic activity of *N. sativa* crude extract, oil or its derivative thymoquinone indicated that both *N. sativa* oil and thymoquinone were able to inhibit the mutagenicity of Cr(VI) in both fish species. The mRNA level of CYP1A2, CYP3A and CYP2E1 was significantly lower in these two groups compared to Cr(VI) alone-treated group. Recently, Badary *et al.* [23] reported that daily intake of TQ after, before or during exposure to benzo(a)pyrene [B(a)P] significantly reduced the chromosomes aberration frequencies (CAs) in bone marrow and damaged cells in male mice compared to the highly clastogenic activity of B(a)P alone. These authors suggested that *N. sativa oil* exerts its anticlastogenic activity by suppressing ROS and hence protects against DNA damage.

A number of naturally occurring compounds have been shown to modulate CYP450 system including induction of specific CYP isozymes and the activation or inhibition of these enzymes [24]. Many mutagens and carcinogens are metabolized by CYP enzymes to either biological inactive metabolites or to chemically reactive electrophilic metabolites that covalently bind to DNA producing mutagenesis and carcinogenesis [25]. The activation of CYP450 enzymes is the most relevant pathway for promutagen. Hence, it seems reasonable to postulate that *N. sativa* oil and its derivative thymoquinone may exert their antigenotoxic effect by modulating the activity of CYP450.

In pathological conditions, reactive oxygen species (ROS) are over produced and result in lipid peroxidation and oxidative damage. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules [26]. Previous studies showed that ROS are one of the important factors in the pathogenesis of Cr (VI)-induced damage [26]. In the present study, Cr(VI) administration induced marked damage to the liver, brain and gills of both fish species. These results were in agreement with those reported by Mohamed [27]. It was reported that Cr(VI) causes severe oxidative stress in tissues manifested as stimulated lipid peroxidation by increasing MDA and decreasing of GSH content in rats [28] and fish [29]. The protective effects of *N. sativa* oil can be attributed to the improvement of the antioxidant status of fish due to an increase in antioxidants or the

presence of free radical scavenging substances [9]. It was also reported that *N. sativa* oil given to sensitized quinea pigs, inhibits free radical generation and increases serum levels of SOD and glutathione [30].

In conclusion: *N. sativa* oil and its thymoquinone extract is effective in inhibiting CrVI-induced mutagenicity in tilapia and zebrafish. This inhibition may be mediated through antioxidant activity and inhibition of CrVI enzymatic activation. This work is of importance as *Nigella sativa* oil and its thymoquinone extract can be used as a natural dietary supplement to counteract the mutagenic effects of Cr(VI) or any other environmental pollutants .

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