African Journal of Food Science Vol. 6(22) pp. 529-534, 26 November, 2012
Available online http://www.academicjournals.org/AJFS
DOI: 10.5897/AJFS12.066
ISSN 1996-0794 ©2012 Academic Journals

Full Length Research Paper

Oxidative and genotoxic effects of Thymoquinone, *Nigella sativa* active compound, in Balb/c mice

Hanene Jrah Harzallah[1]*, Karima Rjiba[2], Aya Maaloul[1], Salwa Abid-Essafi[2] and Touhami Mahjoub[1]

[1]Research Unit of Cancer Biology and Genetics, Hematologic and Autoimmune Diseases, Faculty of Pharmacy, Monastir, Tunisia.
[2]Laboratory for Research on Biologically Compatible Compounds, Faculty of Dentistry, Monastir, Tunisia.

Accepted 27 July, 2012

Nigella sativa **has been traditionally used for the treatment of many ailments. Experimentally, it has been demonstrated that *N. sativa* extracts and Thymoquinone (TQ), the main compound of their volatile oil, possess several biological activities such as antioxidant, anti-inflammatory and hepato-protective properties. To further evaluate the oxidative damage and genotoxicological properties of TQ *in vivo*, this compound was injected with increasing doses by intraperitoneal route to Balb/c mice, and both oxidative and genotoxic effects were tested. Malondialdehyde (MDA) levels and catalase (CAT) activity in liver and kidney were determined as endpoints of oxidative damage, while chromosomal aberrations and the DNA damage index in liver and kidney served as endpoints of genotoxicity. These experiments showed that TQ demonstrated oxidative and genotoxic effects in a concentration dependent manner by inducing significant increase of MDA level and CAT activity from 40 mg/kg body weight (b.w). At high dose (80 mg/kg b.w.), TQ significantly increased chromosomal aberrations and the DNA damage index in liver and kidney. These results contradict the reports indicating antioxidant and anti-genotoxic effects.**

Key words: *Nigella sativa*, Thymoquinone, Malondialdehyde (MDA), catalase, comet test, chromosomic abberations.

INTRODUCTION

In recent years, many studies on natural products and healthy foods with anti-cancer potential have been made due to their relatively nontoxic, inexpensive and available in an ingestive form. More than 25% of drugs used during the last 20 years have been directly derived from plants (Vuorelaa et al., 2004). *Nigella sativa* L. is an annual herbaceous plant which belongs to Ranunculaceae family, and is commonly used traditionally in Middle Eastern folk medicine as a natural remedy for various diseases for over 2000 years (Phillips, 1992). In South Asia, it is known as Kalonji and in the English literature, it is known as black cumin (Ghedira, 2006). It has been particularly used in traditional Arab herbal medicine for the treatment of many diseases and ailments. Recently, many biological activities of *N. sativa* seeds have been reported, including: antibacterial (Ferdous and Islam, 1992), anti-tumor (Worthen et al., 1998), diuretic and hypotensive (Zaoui et al., 2000). The biological activity of *N. sativa* seeds is attributed to its essential oil components (Hajhashemi et al., 2004). The main compounds of this plant are Thymoquinone (TQ) (30 to 48%), p-cymene (7 to 15%), carvacrol (6 to 12%), 4-terpineol (2 to 7%), t-anethole (1 to 4%) and the sesquiterpene longifolene (1 to 8%) (Burits and Bucar, 2000). Pharmacological studies have demonstrated that TQ possesses important properties such as, analgesic and anti-inflammatory (El Gazzar et al., 2006), protection of organs against oxidative damage induced by a variety of free radical generating agents [including carbontetrachloride, cis-platin, doxorubi-

*Corresponding author. E-mail: jrahhanene@yahoo.fr. Tel: +21673461000. Fax: +21673461830.

cin and recently human immunodeficiency virus type 1 (HIV-1) protease inhibitor] (El Gazzar et al., 2006), inhibition of eicosanoid generation and membrane lipid peroxidation (Gali-Muhtasib et al., 2006). Many investigators have shown that the growth inhibitory effects of TQ are specific to cancer cells: significant anti-neoplastic activity was observed against human pancreatic, adenocarcinoma, uterine sarcoma and leukemic cell lines, while it is minimally toxic to normal cells (Gali-Muhtasib et al., 2004b, 2004a, 2006; Edris, 2009). TQ induces p53-independent apoptosis through activation of caspase 8 and other caspases, including caspases 9 and 3, in the caspase cascade. TQ also modulates the Bax/Bcl2 ratio by upregulation of proapoptotic Bax and downregulation of antiapoptotic Bcl2 proteins in p53-null myeloblastic leukemia HL-60 cells during apoptosis (El-Mahdy et al., 2005). Yi et al. (2008) demonstrated that TQ was considered to be a potential drug for cancer therapy by inhibiting tumor angiogenesis and tumor growth. In a recent study (Khalife and Lupidi, 2007), it was showed that under physiological conditions, TQ reacts with glutathione (GSH) or reduced nicotinamide adenine dinucleotide (NADH) and reduced phosphorylated nicotinamide adenine dinucleotide (NADPH) through a spontaneous reaction to produce species (DHTQ-GS or DHTQ) whose antioxidant properties were higher than those of TQ and similar to those of Trolox, a water soluble analogue of vitamin E. The high potency and low systemic toxicity of TQ make it a promising alternative to conventional therapeutic drugs. TQ is a promising compound with significant *in vitro* and *in vivo* antitumor activities against different tumor models (Ivankovic et al., 2006). Furthermore, in a rat, it has been showed that TQ could be useful for treatment of acute respiratory distress syndrome (Isik et al., 2005).

Badary et al. (1998) reported that TQ induced hypoactivity and difficulty in respiration as signs of toxicity at high doses (2 to 3 g/kg), and 24 h after TQ administration, a significant reduction in tissue (liver, kidneys and heart) reduced GSH content was observed. Plasma urea and creatinine concentrations and the enzyme activities of alanine amino transferase (ALT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) were significantly increased.

TQ demonstrated cyto- and genotoxic effects in a concentration dependent manner; it induced significant anti-proliferative effects at 20 µM and acute cytotoxicity. Furthermore, TQ induced significant genotoxicity at concentrations ≥1.25 µM (Khader et al., 2009).

The aim of the present study was (1) to find out whether oxidative damage in kidney and liver of TQ at different concentrations in Balb/c mice and (2) to investigate the possible genotoxicologicals properties of TQ after administration to mice a single of increasing doses (10, 20, 40 and 80 mg/kg b.w). Thus, the Malondialdehyde (MDA) concentrations, catalase (CAT) activity, chromosome aberrations and comet assay *in vivo* were also investigated.

MATERIALS AND METHODS

Chemicals

TQ (> 99% pure), thiobarbituric acid (TBA), trichloroacetic acid (TCA) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used in this study were of analytical grade (Merck, Germany).

Animals' treatments

Blab/c mice were used for *in vivo* study (average body weight (b.w) 22 ± 1.8 g; age, 6 weeks old). These mice were given a standard granulated food and drinking water and were divided into six groups as follows:

Group 0: Mice given water
Group 1: Mice given ethanol/water (4:1)
Group 2: Mice given TQ at 10 mg/kg b.w
Group 3: Mice given TQ at 20 mg/kg b.w
Group 4: Mice given TQ at 40 mg/kg b.w
Group 5: Mice given TQ at 80 mg/kg b.w

A single administration by intra-peritoneal injection of these increasing single doses in 200 µl of solutions was performed. 24 h after treatments, animals were sacrificed by cervical dislocation. Kidney and liver were dissected out. All animal studied were conducted using a protocol approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy of Monastir, Tunisia.

Preparation of liver and kidney extracts

Liver and kidney were homogenized with a Potter (glass-Teflon) in the presence of 10 mM Tris-HCl, pH 7.4 at 4°C and centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was collected for analysis and the protein concentration were determined in liver and kidney extract using Protein BioRad assay (Bradford, 1976).

Evaluation of lipid peroxidation status

Lipid peroxidation was indirectly determined by measuring the production of MDA in the renal and liver extracts following the method described by Yagi (1976). Briefly, 200 µl of liver or kidney extracts were mixed with 150 µl of Tris buffered saline (TBS) (Tris 50 mM and NaCl 150 mM, pH 7.4) and 250 µl TCA–BHT (20% TCA and BHT 1%). The mixture was vigorously mixed and centrifuged at 1500 g for 10 min. 400 µl of the supernatant were added with HCl 0.6 N and 320 µl Tris-TBA (Tris 26 mM and TBA 120 mM), and the content was mixed and incubated 10 min at 80°C. The absorbance was measured at 535 nm. The optic density corresponding to the complex formed with the TBA–MDA is proportional to the concentration of MDA. The concentration in µmol of MDA/mg of proteins is calculated from the absorbance at 535 nm using the molar extinction coefficient of MDA $1.56 \times 105\ M^{-1}\ cm^{-1}$.

Determination of CAT activity

CAT activity was measured in the liver and kidney extracts at 240 nm, 25°C according to Clairbone (1985). Briefly, 20 µl of the hemolysat were added in the quartz cuvette containing 780 µl phosphate buffer and 200 µl of H_2O_2 0.5 M. The activity of CAT was calculated in µmol H_2O_2 consumed/min/mg of protein using the molar extinction coefficient ($0.04\ Mm^{-1}cm^{-1}$).

***In vivo* chromosome aberration assay**

Bone marrow preparation

Bone marrow cells were obtained according to the technique of Yosida and Amano (1965). Briefly, femur and tibia were removed immediately after animal sacrifice and bone marrow was flushed out with KCl solution (0.075 M, 37°C) using a syringe.

The bone marrow cell

Suspension was incubated for 20 min at 37°C and centrifuged at 3500 rpm for 10 min. The supernatant was discarded, the pellet was resuspended in 5 ml of a fixative solution (acetic acid / methanol, 1:3, v/v), centrifuged (3500 rpm for 10 min), and the supernatant was discarded again. This step was repeated 3 times in order to clean the pellet. Finally, the pellet was resuspended in 1 ml of the above fixative solution for chromosome preparation.

Chromosome preparation

Chromosomes were prepared as reported by Evans et al. (1960). Cell suspensions were dropped on glass slides giving smears that were dried for 5 s, then air-dried for conservation at room temperature and/or directly stained with Giemsa. Giemsa working solution was freshly prepared [4 ml in 100 ml of phosphate buffer (0.15 M, pH 7.2)]. Slides were left for 15 min in this staining solution, then rinsed with water and allowed to dry at room temperature

Slide analysis

The slides were examined under 100× magnifications using an optical microscope (Carl Zeiss, Oberkochen, Germany). 300 well-spread metaphases were analyzed per group for abnormalities. Metaphases with chromosome breaks, gaps, ring, and centric fusion (Robertsonian translocation) were recorded and expressed as percentage of total metaphases per group.

Comet assay

The alkaline comet assay was carried out as described by Tice et al. (2000) with minor modifications. Each piece of liver and kidney were placed in 0.5 ml of cold phosphate-buffered saline (PBS) and finely minced in order to obtain a cellular suspension. Liver and kidney cell suspensions (5 µl) were embedded in 95 µl of 0.75% low melting point agarose (Gibco BRL) and spread on agarose-precoated microscope slides. After solidification, slides were transferred to either PBS or 0.20 mM freshly prepared H_2O_2 solution for 5 min at 4°C (Pereira et al., 2008). Slides were washed 3 times with PBS and placed in lysis buffer (2.5 M NaCl, 100 mM ethylene diamine tetraacetic acid (EDTA) and 10 mM Tris, pH 10.0), with freshly added 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (DMSO) for 48 h at 4°C. The slides were subsequently incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min at 4°C. An electric current of 300 mA and 25 V (0.90 V/cm) was applied for 15 min to perform DNA electrophoresis. The slides were then neutralized (0.4 M Tris, pH 7.5), stained with silver and analyzed using a microscope. Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed from each animal. Cells were also visually scored according to tail size into five classes ranging from undamaged (0) to maximally damaged (4), resulting in a single DNA damage score to each animal, and consequently to each studied group. Therefore, the damage index can range from 0 (completely undamaged, 100 cells × 0) to 400 (with maximum damage, 100 × 4) (Picada et al., 2003).

Statistical analysis

The results are expressed as means ± SD and statistical significance was determined by one-way analysis of variance (ANOVA). In all comparisons, $P < 0.05$ was considered as indicating statistical significance.

RESULTS

During the experiments, there were no changes in body, liver and kidney weights of mice treated with TQ as compared to control animals.

Induction of lipid peroxidation

Results of the effect of the TQ on the induction of lipid peroxidation in liver and kidney extracts as determined by MDA level are shown in Figure 1.

At 40 and 80 mg/kg b.w, TQ induced a significant increase in MDA formation as compared to control groups in both liver and kidney extracts. Thus, for liver extracts, the MDA level increases from a basal level of 5.52 ± 0.15 µM/mg of protein to reach 15.06 ± 1.56 µM/mg of protein. Similar results were found with kidney extracts, it increased from 6.2 ± 0.13 to achieve 9.18 ± 0.08 as compared to the control group ($p < 0.05$).

CAT activity

Figure 2 shows the effect of the different TQ concentrations in CAT activity. It induced a marked increase in CAT activity in both liver and kidney extracts. At 10 mg/kg b.w. of TQ, the basal level of CAT activity decreased by 5 and 11.9% in liver and kidney, respectively. But, from the dose 40 mg/kg b.w. of TQ, the CAT activity increased in liver and kidney in a dose-dependent way.

Induction of chromosome aberrations

The results of the structural chromosomal aberrations test showed centric fusions, gaps, rings and chromosomal breaks (Table 1). Animals (5 per group) treated with 40 and 80 mg/kg b.w. of TQ showed a significant increase in chromosome aberrations, mainly centric fus-ions and chromosome rings, in bone marrow cells. No significant differences were observed in the group treated with 10 mg/kg b.w. as compared to Group 1 (6.33 ± 1.32 and 7.34 ± 0.86). However, Group 3 showed a significant increase in ring only.

DNA damage

TQ increased the DNA damage index in liver and kidney in a dose-dependent way (Table 2) essentially the high-

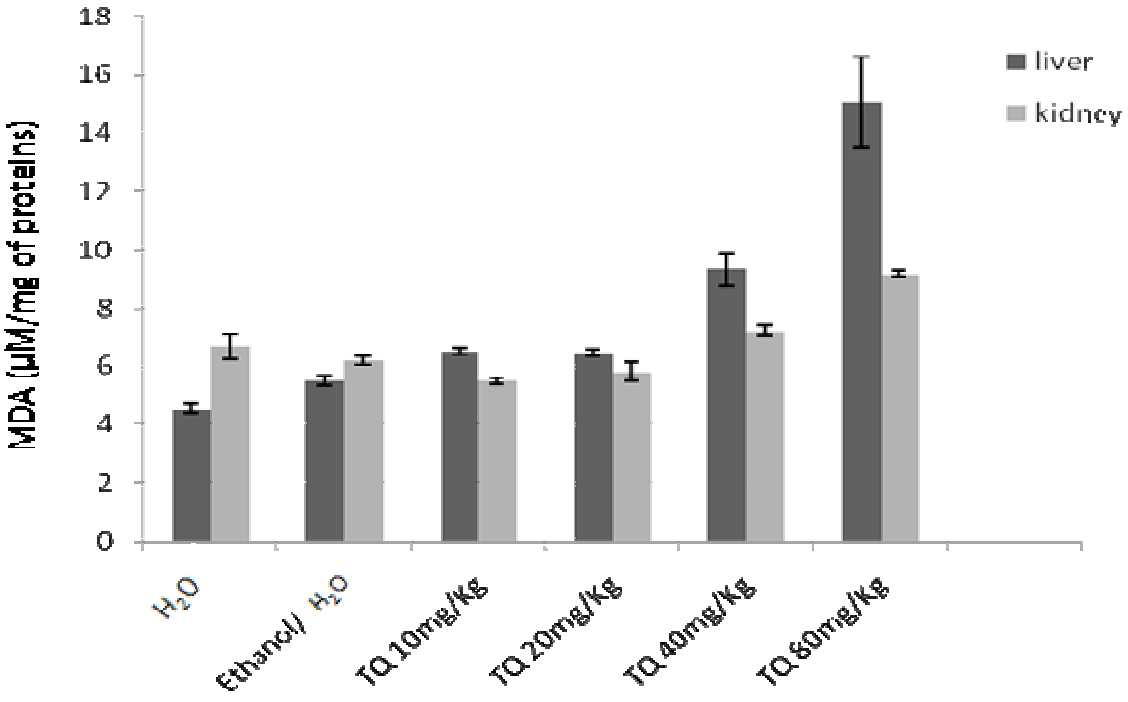

Figure 1. Lipid peroxydation as determined by MDA level in liver and kidney of Balb/c mice treated with different concentration of TQ. Data represented as means ± SD (5 independent experiments per group). * $P < 0.05$, statistically significant difference from the control group (Group 1) (ANOVA Tukey' test). For TQ 40 and TQ 80 mg/kg, $P < 0.05$, statistically significant difference from the control group (Group 1) (ANOVA Tukey' test).

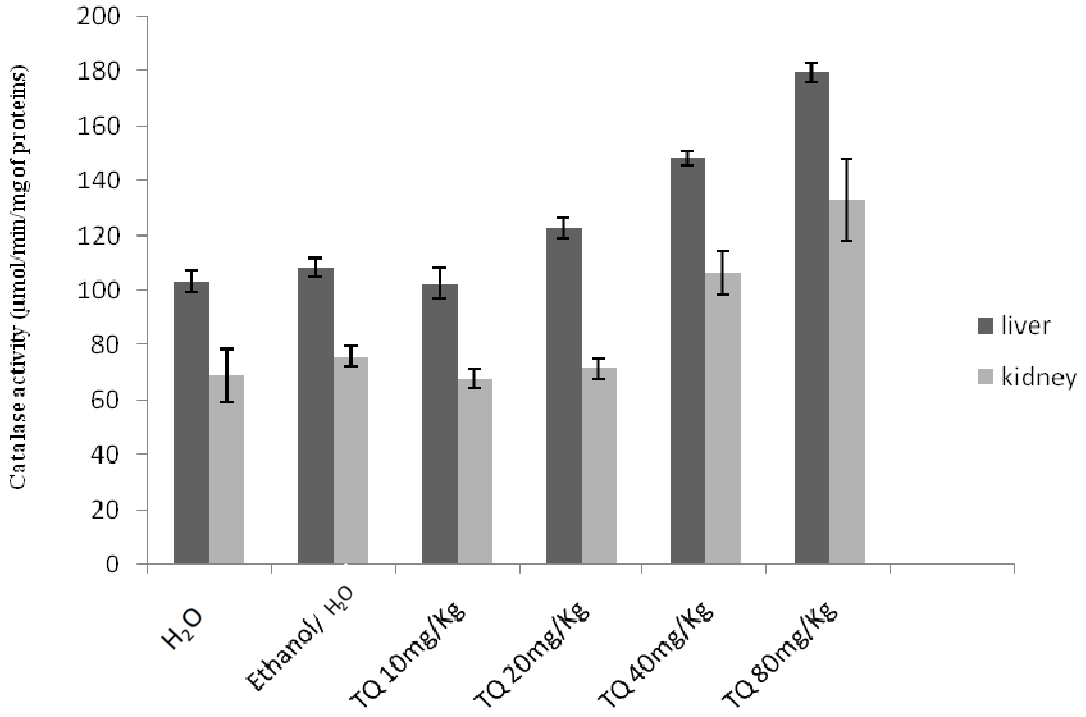

Figure 2. Effect of TQ different concentration on CAT enzyme activity in mice liver and kidney. Data represented as means ± SD (5 independent experiments per group). * $P < 0.05$, statistically significant difference from the control group (Group 1) (ANOVA Tukey' test). For TQ 40 and TQ 80 mg/kg, $P < 0.05$, statistically significant difference from the control group (Group 1) (ANOVA Tukey' test).

Table 1. Percentage of different type of chromosomal damage induced by different concentration **of** TQ.

Group	Ring	Centric fusion	Break	Gap	Total
Group 0 (Water)	1.33 ± 0.58	2.33 ± 1.53	0.33 ± 0.58	1.33 ± 0.58	5.32 ± 0.81
Group 1 (Ethanol/water)	1.67 ± 1.25	1.66 ± 1.53	1.33 ± 1.25	1.67 ± 1.25	6.33 ± 1.32
Group 2 (TQ 10 mg/kg b.w)	2.33 ± 1.25	1.67 ± 0.47	1.67 ± 1.25	1.67 ± 0.47	7.34 ± 0.86
Group 3 (TQ 20 mg/kg b.w)	6.33 ± 1.25*	2.67 ± 0.47	1.33 ± 0.47	1 ± 0.82	11.33 ± 0.75*
Group 4 (TQ 40 mg/kg b.w)	8.67 ± 1.25*	6.33 ± 1.70*	5 ± 2.45	1 ± 0.82	21 ± 1.55*
Group 5 (TQ 80 mg/kg b.w)	9 ± 0.82*	8.33 ± 0.94*	2.67 ± 2.05	2.67 ± 1.8	22.67 ± 1.42*

Data represented as means ± SD (5 independent experiments per group). * $P < 0.05$, Statistically significant difference from the control group (Group 1) (ANOVA Tukey' test).

Table 2. DNA damage index analyzed in liver and kidney from mice treated with different concentration of TQ in liver and kidney on Balb/c mice.

	Group 0 (Water)	Group 1 (Ethanol/water)	Group 2 (TQ 10 mg/kg b.w)	Group 3 (TQ 20 mg/kg b.w)	Group 4 (TQ 40 mg/kg b.w)	Group 5 (TQ 80 mg/kg b.w)
Liver	125.33 ± 8.73	139.66 ± 13.57	102.33 ± 8.65	136.66 ± 11.14	155 ± 7.11	295.33 ± 12.39*
Kidney	111.33 ± 14.38	112.33 ± 20.67	97.33 ± 10.27	108.33 ± 13.88	138.33 ± 19.48	182 ± 9.93*

Damage index, can range from 0 (completely undamaged 100 cells×0) to 400 (with maximum damage 100 cells × 4). * $P < 0.05$, Statistically significant difference from the control group (Group 1) (ANOVA Tukey' test).

est dose. It increased significantly in comparison to the control group from 139.66 ± 13.57 to 295.33 ± 12.39 and from 112.33 ± 20.67 to 182 ± 9.93 in liver and kidney, respectively. The DNA damage varied insignificantly both in liver and kidney in Groups 2, 3 and 4.

DISCUSSION

Oxidative damage is among the most potent and omnipresent threat faced by any living organism. Intracellular accumulation of reactive oxygen species can arise from toxic insults and can perturb the cell's natural antioxidant defense system resulting in damage to all major classes of biological macromolecules. During the last decades, the oxidative stress has been pointed out as major component of several biological and pathological processes like aging, inflammation, carcinogenesis and several other diseases including Parkinson's, Huntington's, etc. (Calabrese et al., 1998). Therefore, arising attention is given to the study of natural products, which may counteract the detrimental effects of oxidative stress and prevent multiple human diseases. In this line, different types of fruits and vegetables have been re-evaluated and recognized as valuable sources of nutraceuticals. Generally, antioxidant and antimutagenic effects of medicinal plant extracts or their constituents are evaluated *in vitro* applying bioassays such as *Salmonella typhimurium* (Ferrer et al., 2001) or bone marrow cells, spleenocytes and human lymphocytes (Aboul-Ela, 2002), TQ was the bioactive constituent of the volatile oil of black seed (El-Dakhakhany, 1963). It exerts antioxidant effects and inhibits inflammation in animal models and cell culture systems. The present investigation demonstrated oxidative and genotoxic effects of TQ in a concentration dependant manner. The oxidative effect is evidenced by a significant increase of MDA level and CAT activity in both liver and kidney at doses of 40 and 80 mg/kg. TQ, like other quinone compounds, can be considered to be a redox-cycler which is metabolized *in vivo* to hydroquinones or semiquinone radicals by cellular oxido-reductases leading to the production of reactive oxygen species. Via this mechanism, TQ could lead to adverse effects and thus, be also responsible for the effects of aqueous extracts of *N. sativa* found in primary rat hepatocytes (Khader et al., 2009).

These results are in line with previous reports which showed that high doses of TQ cause depletion of cellular glutathione (Badary et al., 1998). Furthermore, TQ caused concentration dependent genotoxic effects in Balb/c liver and kidney; it develops an increase of the frequency of chromosomal aberration (Table 1) and the DNA damage index (Table 2). The increase of CAT is significant at 80 mg/kg. Similarly, the increase of the DNA damage index is significant at the highest concentration used (80 mg/kg). It is acknowledged that an increase in the frequency of chromosomal aberrations in bone marrow cells and consequently in peripheral blood lymphocytes is associated with an increased overall risk of cancer (Hagmar et al., 1994). Most of the chromosomal aberrations observed in the cells are lethal, but there are many other aberrations that are viable and cause genetic effects, either somatic or inherited (Swierenga et al., 1991). More studies have to be performed before TQ can be

developed into a drug for the potential treatment of various carcinomas and inflammatory disorders. Thus, clinical trials could be made with this agent with patients to enrich TQ effects data in humans and to enhance its beneficial effect (Harzallah et al., 2012).

Conclusion

Our results showed that TQ at higher concentrations increased oxidative stress, which can cause DNA damage in liver and kidney. As TQ was discussed as therapeutic drug for treatment of various diseases including cancer, so its medicinal use requires further knowledge on its pharmaco-dynamics and metabolism.

ACKNOWLEDGEMENTS

This research was funded by the Tunisian Ministry of Scientific Research and Technology through the Laboratory for Research on Biologically Compatible Compounds, Faculty of Dentistry and the Research Unit of cancer biology and genetics, hematologic and autoimmune diseases, Faculty of Pharmacy, Monastir, Tunisia.

REFERENCES

Aboul-Ela EI (2002). Cytogenetic studies on Nigella sativa seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. Mutat. Res. 516:11-17.

Badary OA, Al-Shabanah OA, Al-Gharably NM, Elmazar MM (1998). Effect of Cremophor EL on the pharmacokinetics, antitumor activity and toxicity of doxorubicin in mice. Anticancer Drugs 9:809-815.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Burits M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323-288.

Calabrese L, Tradati N, Nickolas TL, Giugliano G, Zurrida S, Scully C, Boyle P, Chiesa F (1998). Cancer screening in otorhinolaryngology. Oral Oncol. 34:1-4.

Clairbone A (1985). Catalase activity. Handbook of Methods for Oxygen Radical Research. CRC Press, Boca. Raton. pp. 283–284.

Edris AE (2009). Anti-cancer properties of *Nigella* spp. essential oils and their major constituents, thymoquinone and beta-elemene. Curr. Clin. Pharmacol. 4:43-46.

El-Dakhakhany M (1963). Studies on the chemical constitution of Egyptian *N. sativa* L. seeds. Planta Medica. p. 11.

El-Mahdy A, Qianzheng Z, Qi-En W, Gulzar W, Wani AA (2005). Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. Int. J. Cancer. 117:409-417.

El Gazzar M , El Mezayen R , Nicolls MR , Marecki JC , Dreskin SC (2006). Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. Int. Immunopharmacol. 6:1135-42.

Evans EP, Breckon G, Ford CE (1960). An air drying method for meiotic preparation from mammalian tests. Cytogenetics p. 3.

Ferdous AJ, Islam SN (1992). *In vitro* antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug- resistant isolates of *Shigella* spp. and isolates of *Vibrio cholerae* and *E. coli*. Phytother. Res. p.6.

Ferrer M, Sánchez-Lamar A, Fuentes JL, Barbé J, Llagostera M (2001). Studies on the antimutagenesis of *Phyllanthus orbicularis*: mechanisms involved against aromatic amines. Mutat. Res. 498:99-105.

Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, Schneider-Stock R (2004). Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. Int. J. Oncol. 25:857-866.

Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, Schneider-Stock R (2006). Thymoquinone: a promising anti-cancer drug from natural sources. Int. J. Biochem. Cell Biol. 38:1249-53.

Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, Schneider-Stock R (2004). Molecular pathway for thymoquinone-induced cell-cycle arrest and apoptosis in neoplastic keratinocytes. Anticancer Drugs 15:389-399.

Ghedira K (2006). Nigelle cultivée: *Nigella sativa* L. (Ranunculaceae). Phytothérapie. p. 4.

Hagmar L, Brøgger A, Hansteen IL, Heim S (1994). Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res. 54:2919-2922.

Hajhashemi V, Ghannadi A, Jafarabadi H (2004). Black cumin seed essential oil, as a potent analgesic and antiinflammatory drug. Phytother. Res. 18:195-199.

Isik AF, Kati I, Bayram I, Ozbek H (2005). A new agent for treatment of acute respiratory distress syndrome: thymoquinone. An experimental study in a rat model. Eur. J. Cardiothorac. Surg. 28:301-305.

Ivankovic S, Stojkovic R, Jukic M, Milos M, Milos M, Jurin M (2006). The antitumor activity of thymoquinone and thymohydroquinone *in vitro* and *in vivo*. Exp. Oncol. 28:220-224.

Khader M, Bresgen N, Eckl PM (2009). *In vitro* toxicological properties of thymoquinone. Food. Chem Toxicol. 47:129-133.

Khalife KH, Lupidi G (2007). Nonenzymatic reduction of thymoquinone in physiological conditions. Free Radic. Res. 41:153-161.

Pereira AD, de Andrade SF, de Oliveira Swerts MS, Maistro EL (2008). First *in vivo* evaluation of the mutagenic effect of Brazilian green propolis by comet assay and micronucleus test. Food. Chem. Toxicol. p. 46.

Phillips JD (1992). Medicinal plants. Biologist. p. 39.

Picada JN, Flores DG, Zettler CG, Marroni NP, Roesler R, Henriques JA (2003). DNA damage in brain cells of mice treated with an oxidized form of apomorphine. Brain. Res. Mol. Brain. Res. 114:80-85.

Swierenga SH, Gilman JP, Brillinger RL, Nestmann ER (1991). Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: study design. Mutat. Res. 246:227-233.

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000). Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environmental and Molecular Mutagenesis. p. 35.

Vuorelaa P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, Vuorela H (2004). Natural products in the process of finding new drug candidates. Curr. Med. Chem. 11:1375-1389.

Worthen DR, Ghosheh OA, Crooks PA (1998). The *in vitro* anti-tumor activity of some crude and purified components of blackseed, *Nigella sativa* L. Anticancer Res. 18:1527-1532.

Yagi K (1976). A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med. 15:212-216.

Yi T, Cho SG, Yi Z, Pang X, Rodriguez M, Wang Y, Sethi G, Aggarwal BB, Liu M. (2008). Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol. Cancer. Ther. 7:1789-1796.

Yosida TH, Amano K (1965). Autosomal polymorphism in laboratory bred and wild Norway rats, *Rattus norvegicus*, found in Misima. Chromosoma 16:658-667.

Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M (2000). [Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat]. Therapie 55:379-382.