The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.36, No.5, 531-537, 2011

Original Article

Olive (*Olea europaea* L.) leaf extract counteracts genotoxicity and oxidative stress of permethrin in human lymphocytes

Hasan Türkez and Başak Toğar

Department of Biology, Faculty of Science, Atatürk University, 25240, Erzurum, Turkey

(Received June 7, 2011; Accepted July 6, 2011)

ABSTRACT — The aim of this study was to investigate the protective effects of olive leaf extract (OLE) on genotoxicity and oxidative damage in cultured human blood cells treated with permethrin (PM) in the presence of a rat liver S9 mix containing cytochrome P 450 enzymes. Anti-genotoxic activities of OLE were studied using sister chromatid exchange (SCE) and chromosome aberration (CA) tests and furthermore total antioxidant capacity (TAC) and total oxidative status (TOS) were examined to determine the oxidative damage. Our results clearly revealed that treatment with PM (200 mg/l) alone increased SCE and CA rates and TOS level, decreased TAC level in cultured human blood cells. The OLE alone at the all tested doses did not induce any significant changes in the genotoxicity endpoint. However OLE leads to increases of plasma TAC level in vitro. OLE starts showing this positive effect at 100 mg/l. The combined treatment showed significant improvements in cytogenetic and biochemical parameters tested. Moreover, this improvement was more pronounced in the group received the high dose of the OLE. It could be concluded that the ethanol extract of OLE induced its genoprotective effect via the increase in the antioxidant capacity, inhibition of oxidative stress and scavenging of free radicals.

Key words: Antioxidant capacity, Genoprotective effect, Genotoxicity, Oxidative damage, Whole blood culture

INTRODUCTION

Increasing use of pesticides and their potential toxic effects among humans and animals warrant a heightened awareness about these chemicals (David *et al*., 2007; Kapoor *et al*., 2010) Hence, pesticides still pose a major problem in toxicology (Askar *et al*., 2011). The previous reports showed that permethrin (PM) was a highly toxic synthetic pyrethroid pesticide widely used in agriculture aquatic organisms and vector control programs (Vadhana *et al*., 2010; Fojut and Young, 2011; Srivastava *et al*., 2006). Reproductive toxic (Zhang *et al*., 2008), immunotoxic (Jin *et al*., 2010), neurotoxic (De Micco *et al*., 2010) and cardiotoxic (Vadhana *et al*., 2010) effects of PM were reported. PM was also shown to induce DNA damage on rat heart cells (Vadhana *et al*., 2010). Furthermore, potential carcinogenicity of PM was ascertained in human nasal mucosal cells (Tisch *et al*., 2002). In addition Hu *et al*. (2010) demonstrated that PM induced oxidative stress in rat adrenal pheochromocytoma (PC12) cells.

Olive leaf from *Olea europaea* L. the olive tree, is native to the Mediterranean and has been known for its medicinal properties since ancient times. Since these leaves contain many potentially bioactive compounds, they may have antioxidant properties (Khayyal *et al*., 2002). At the same time olive leaves have been heavily exploited for the prevention or the treatment of hypertension, carcinogenesis, diabetes, atherosclerosis and so many other traditional therapeutic uses (Mijatovic *et al*., 2011; Bouallagui *et al*., 2011). Lee and Lee (2010) reported that the combination of olive leaf extract phenolics possessed antioxidant activity. Poudyal (Poudyal *et al*., 2010) found that an OLE containing polyphenols such as oleuropein and hydroxytyrosol reversed the chronic inflammation and oxidative stress by diet-induced obesity and diabetes in cardiovascular, hepatic, and metabolic symptoms in rats.

Recent studies focused on exploring protective agents, such as vitamins C and E, coenzyme Q(10) and glutathione, against permethrin toxicity (Vontas *et al*., 2001;

Correspondence: Başak Toğar (E-mail: basaktogar@gmail.com)

Gabianelli *et al*., 2004; Falcioni *et al*., 2010) To our best knowledge, the effects of OLE against the toxicity of PM have not been investigated. Therefore, in this investigation it was aimed to explore the role of OLE on PM-induced genotoxicity in human lymphocyte cells by using SCE and CA tests. In addition, TAC and TOS were measured to assess oxidative alterations in cultured human blood cells.

MATERIALS AND METHODS

Experimental design

Olive leaves were collected from the Balıkesir-Edremit region in Turkey in June 2010. Olive leaf samples were handpicked randomly from the trees. They were washed thoroughly in tap water, dried under shade and powdered to coarse particles. After crushing, 25 g of powdered leaves was extracted. The extract was manufactured from the dried leaves of olive applying ethanol extract procedure. The powder was defatted with 3,000 ml of ethanol overnight at room temperature with constant stirring. The yield of the ethanol extract was found to be 0.75% (w/v). The extract was dried in a desiccator and it was referred to as ethanol extract. It was diluted with 2% Tween-80 to desired concentrations and used for the experiments. Permethrine (Cas No 52645-53-1; $C_{21}H_{20}Cl_2O_3$) was also obtained from Riedel-de Haen® Company (Berlin, Germany).

About 8 ml whole heparinized human blood from five healthy non-smoking female donors between the ages 27 and 28 with no history of exposure to any genotoxic agent was used in our experiments. In all the volunteers involved in this study, hematological and biochemical parameters were analyzed and no pathology was detected. Experiments conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). The study protocol was also approved by the local ethical committee. The heparinize blood (0.5 ml) was cultured in 6 ml of culture medium (Chromosome Medium B, Biochrom® Leonorenstr. 2-6.D, Berlin, Germany) with 5 µg ml of phytohemagglutinin (Biochrom®). A various concentrations (50, 100, 200 and 400 mg/l) extracts of OLE and PM (200 mg/l) were added separately or together to culture tubes just before the incubation period. The doses were selected according to the literature data (Undeğer and Başaran, 2005; Ramkumar *et al*., 2010). SCE and CA rates were assessed in peripheral lymphocytes in the presence of a supplemented liver fraction (*S9 mix*). For S9 mix preparation, Sprague-Dawley healthy rats were used. The cultures without extracts and PM were studied as control$^-$ group Mitomycin C (10^{-7} M) (Vijayalaxmi *et al*., 1996) was used as the positive control in SCE and CA assays. Likewise, ascorbic acid (10 µM) (Turkez, 2011) and hydrogen peroxide (25 µM) (Benhusein *et al*., 2010) were also used as the positive controls in TAC and TOS analysis, respectively.

SCE assay

With the aim of providing successive visualization of SCEs, 5-bromo-2'-deoxyuridine (Sigma®) was added at culture initation. The cultures were incubated in complete darkness for 72 hr at 37°C. Exactly 70 hr and 30 min after beginning the incubations, demecolcine (N-diacetyl- N-methylcolchicine, Sigma®) was added to the cultures. After hypotonic treatment (0.075 M KCl), followed by three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled, grease-free microscopic slides, air-dried, aged for three days, and then differentially stained for the inspection of the SCE rate according to fluorescence plus Giemsa (FPG) procedure. For each treatment condition, well-spread twenty five second division metaphases containing 42-46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell (Geyikoglu *et al*., 2007).

CAs assay

Exactly 70 hr after beginning the incubations, 0.1 ml of colchicine (0.2 mg/ml, Sigma) was added to the culture flask for 2 hr. Hypotonic treatment and fixation were performed. To prepare slides, 3-5 drops of the fixed cell suspension were dropped on a clean slide and air-dried. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. For each treatment, 30 well-spread metaphases were analyzed to detect the presence of chromosomal aberrations. Criteria to classify the different types of aberrations (chromatid or chromosome gap and chromatid or chromosome break) were in accordance with the recommendation of Environmental Health Criteria (EHC) 46 for environmental monitoring of human populations (IPCS, 1985).

TAC and TOS analysis

The major advantage of TAC test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound (Kusano and Ferrari, 2008). In this test, antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is tradition-

ally named as Trolox Equivalent that is a vitamin E analog [30]. Since the measurement of different oxidant molecules separately is not practical and their oxidant effects are additive, the total oxidant status (TOS) of a sample is measured and this is named total peroxide (TP), serum oxidation activity (SOA), reactive oxygen metabolites (ROM) or some other synonyms. In TOS assay performed here, oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./l) (Erel, 2004, 2005). The automated TAC and TOS assays were carried out by commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey) on plasma samples of PM and OLE treated cultures for 2 hr.

Statistics

The statistical analysis of experimental values in the SCE, CA, TAC and TOS analysis was performed by one way analysis of variance (ANOVA) and Duncan's test using the S.P.S.S. 13.0 software. The level of 0.05 was regarded as indicative of statistical significance for all tests.

RESULTS

The statistically significant elevations of SCE and CA were observed after exposure to PM for 72 hr. When assayed *in vitro* on the lymphocyte cells, the SCE/cell and CA/cell values for PM-treated cells ranged from 1.6 and 2.9 fold higher than that for the control$^-$ cells, respectively. On the other hand, OLE (at all tested concentrations) did not induce significant ($p < 0.05$) increases of the observed SCE and CA rates as compared to control group. OLE at all concentrations (except for 50 mg/l) reduced the number of PM-induced SCE and CA formation (Figs. 1, 2, 3 and 4). Moreover 200 and 400 mg/l of OLE completely inhibited the PM caused genetic damages.

Table 1 shows the effect of OLE and PM on oxidant status in human whole blood cultures were determined by TAC and TOS analysis. As shown in Table 1, the TAC value decreased by 26.9% with the addition of PM while TOS value increased by 18.0%. In contrast to, OLE did not alter the TOS level but increased the TAC level by about 6.3-38.1%. Besides, the OLE had dose dependent inhibitory effects on oxidative damage in human blood cells by PM.

DISCUSSION

The data obtained from the present study clearly revealed that PM induced genotoxic and oxidative damage in cultured human blood cells. Similarly to our findings, a few reports indicated in vitro and in vivo PM genotoxicity. Institóris *et al*. (1999) investigated the genotoxic effects of PM by structural and numerical CA in bone marrow cells. They showed that PM increased the number of numerical CA in rats. In another in vivo report, PM significantly increased the DNA damage in a concentration-dependant manner in healthy human lymphocytes (Undeğer and Başaran, 2005). Furthermore, PM was tested for its in vitro ability to induce CA, MN and SCE in cultured human peripheral blood lymphocytes and PM was characterized as S-phase independent agent with greater potential for inducing CA than SCE (Barrueco *et al*., 1992, 1994). Likewise Undeğer and Başaran (2005) found that PM significantly increased DNA damage in human lymphocytes. Possible genotoxic effects in primary human nasal mucosal cells were also investigated by Tisch *et al*. (2002). Their findings indicated a significant genotoxic response that was concentration dependent thereby they provided evidence for the potential carcinogenicity of PM to these cells. PM increased the MN frequency in human blood cultures (Surrallés *et al*., 1995). In contrast to those results, Djelic and Djelic (2000) reported that PM was non-genotoxic by using MN assay in cultured human lymphocytes. The present findings also indicated that PM caused oxidative stress on blood cells in human lymphocytes. Because, increases in TOS levels and decreases in TAC levels were determined in PM alone treated cultures as compared to control group. In accordance with our findings, recent investigations clearly indicated that PM-induced oxidative stress leads to biochemical and functional changes in organisms (Hu *et al*., 2010; Nasuti *et al*., 2007; Gabbianelli *et al*., 2009; Issam *et al*., 2011).

The present study also demonstrated that the reduction of PM induced oxidative and DNA damages were caused by the protective effect of OLE. O'Brien *et al*. (2006) provided evidence that non-nutrient dietary constituents could act as significant bioactive compounds and that plant extracts, such as OLE, strongly protect against oxidative stress. Moreover, OLE demonstrated strong antioxidant potency and inhibited cancer and endothelial cell

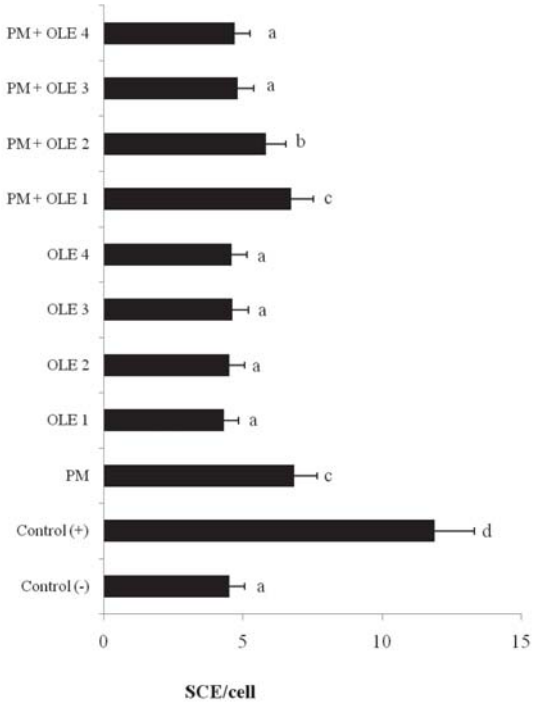

Fig. 1. Sister chromatid exchanges of human lymphocytes (n = 5) treated with PM and OLE for 72 hr. Values are presented as mean ± S.D.; n = 5, bars shown by the same letter are not significantly different from each other at a level of 5% using Duncan's test, Control^{-}: negative control; Control^{+}: positive control (MMC: mitomycin C (10^{-7} M); PM: 200 mg/l of permethrin; OLE1: 50 mg/l olive leaf ethanol extract; OLE2: 100 mg/l olive leaf ethanol extract; OLE3: 200 mg/l olive leaf ethanol extract; OLE4: 400 mg/l olive leaf ethanol extract.

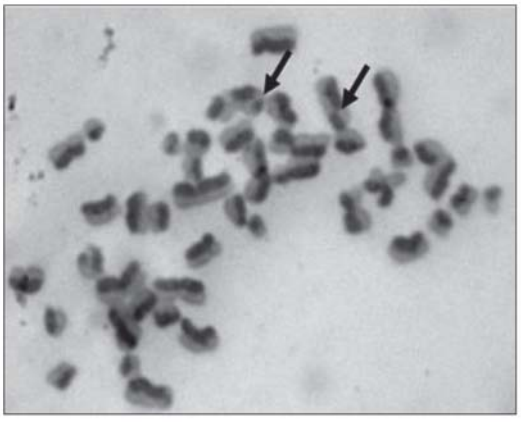

Fig. 3. A sample metaphase from PM (200 mg/l) treated culture. (Arrows show sister chromatid exchange points). x 1000.

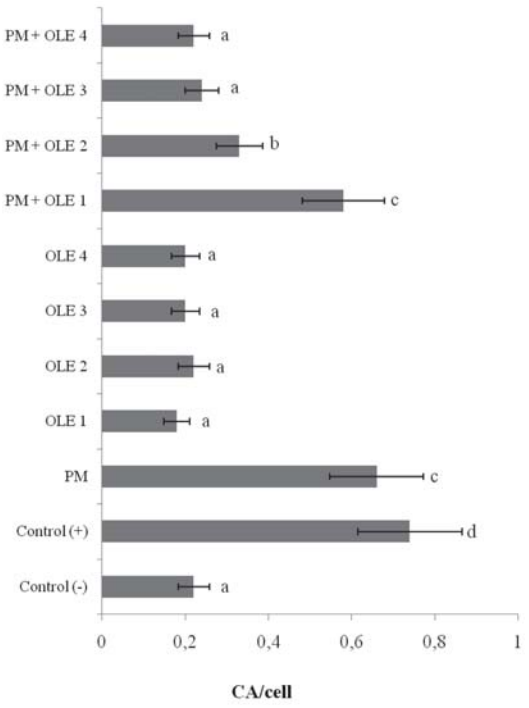

Fig. 2. Chromosome aberrations of human lymphocytes (n = 5) treated with PM and OLE for 72 hr. (Abbreviations are as in Fig. 1).

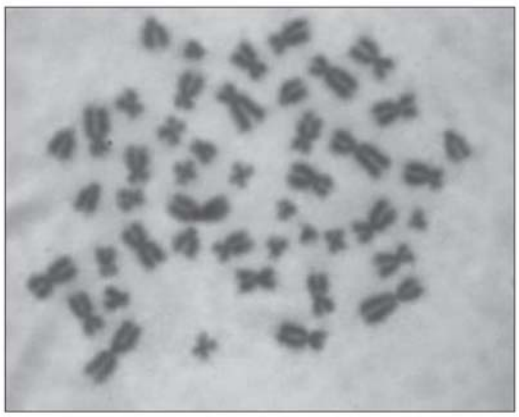

Fig. 4. A sample metaphase from PM (200 mg/l) plus 400 mg/l of OLE-treated culture. x 1000.

proliferation at low micro molar concentrations (Goulas *et al*., 2009).

OLE exhibited potent free radical scavenging capacity in pheochromocytoma (PC12) cells and streptozotocin-induced diabetic rats (Kaeidi *et al*., 2011). The anti-

Table 1. The effect of OLE against PM in the TAC and TOS analysis in plasma samples of cultured human blood cells.

Treatments	TAC (mmol Trolox Equiv./l)	TOS (μmol H_2O_2 Equiv./l)
$Control^-$	6.3 ± 0.6	11.8 ± 2.9
$Control^+$	13.9 ± 1.1**	40.3 ± 5.1**
PM	4.6 ± 0.4**	16.4 ± 3.4**
OLE 1	6.7 ± 0.6	11.2 ± 4.2
OLE 2	7.7 ± 0.5**	11.1 ± 3.9
OLE 3	8.1 ± 0.8**	11.5 ± 3.7
OLE 4	8.7 ± 0.6**	10.9 ± 4.6
PM + OLE 1	5.0 ± 0.5**	14.4 ± 4.7**
PM + OLE 2	5.6 ± 0.7**	13.8 ± 4.4**
PM + OLE 3	6.1 ± 0.5	12.3 ± 4.6
PM + OLE 4	6.4 ± 0.8	11.6 ± 5.2

Values are presented as mean ± S.D.; n = 5, bars shown by the same letter are not significantly different from each other at a level of 5% using Duncan's test, $Control^-$: negative control; $Control^+$ for TAC analysis: positive control is Ascorbic acid (10^{-7} M); $Control^+$ for TOS analysis is H_2O_2 (25 μM). The abbreviations are as in Fig. 1.

oxidant activity of OLE was also determined in human leukemic cell line (Jurkat) (Fares *et al*., 2011). Again, OLE decreased the intracellular levels of reactive oxygen species (ROS) in human embryonic fibroblasts and H_2O_2-exposed in insulin secreting β-cells (Katsiki *et al*., 2007; Cumaoglu *et al*., 2011). Thus, OLE could modulate the PM-induced genetic and oxidative damage by preventing free radical generation or by stimulating components of the antioxidant defense system. As a matter of fact, OLE was found to have free oxygen radicals and lipoperoxyradicals scavenging capacity and, anti-clastogenic activity since it contain polyphenolic compounds, mainly catechol groups (oleuropein, hydroxytyrosol, verbascoside, rutin, luteolin) against in vivo X-ray irradiation treatments (Benavente-García *et al*., 2002). In an *in vivo* study, it was determined that oleuropein-rich extracts from olive leaves increased the serum antioxidant potential and the hepatic catalase (CAT) and superoxide dismutase (SOD) enzyme activities in rats (Jemai *et al*., 2008). Likewise recent studies revealed the relationship between the major phenolic compound oleuropein and its pharmacological activities including antioxidant, anti-inflammatory, and anti-cancer activities (Omar, 2010). Hydroxytyrosol in leaves from *O. europaea* was reported to have powerful antioxidant activity (Zrelli *et al*., 2011). Verbascoside reduced the production of superoxide radicals in human myelomonocytic leukaemia (THP-1) cells (Speranza *et al*., 2010). Rutin pretreatment prevented cisplatin-induced deteriorative effects by reduction of increased oxidative stress as well as caspase-3, TNF-α and NFκB protein expression levels (Arjumand *et al*., 2011). The flavonoid luteolin has been also shown to possess direct antioxidant activity that involved changes in SOD activity, the malondialdehyde (MDA) content and expression of Heme Oxygenase-1 (HO-1) protein (Wang *et al*., 2011).

OLE acted as a genoprotective and antioxidant agent against PM-induced toxicity. It was concluded that OLE can be considered a potential candidate to protect all cells against the deleterious effect of oxidative DNA damages. Furthermore, evaluation of *in vitro* antigenotoxic and antioxidant activities of olive leaf extract has also provided interesting results that might be beneficial for the use of this plant in clinical trials.

ACKNOWLEDGMENT

The authors are grateful to volunteers for blood samples and Mr. S. Yesilyurt for providing olive leaf samples.

REFERENCES

Arjumand, W., Seth, A. and Sultana, S. (2011): Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NFκB, TNF-α and caspase-3 expression in wistar rats. Food. Chem. Toxicol., (in press), (doi:10.1016/j.fct.2011.05.012).

Askar, K.A., Kudi, A.C. and Moody, A.J. (2011): Spontaneous reactivation and aging kinetics of acetylcholinesterase inhibited by dichlorvos and diazinon. J. Toxicol. Sci., **36**, 237-241.

Barrueco, C., Herrera, A., Caballo, C. and de la Peña, E. (1992): Cytogenetic effects of permethrin in cultured human lymphocytes. Mutagenesis., **7**, 433-437.

Barrueco, C., Herrera, A., Caballo, C. and de la Peña, E. (1994) : Induction of structural chromosome aberrations in human lymphocyte cultures and CHO cells by permethrin. Teratog. Carcinog. Mutagen., **14**, 31-38.

Benavente-García, O., Castillo, J., Lorente, J. and Alcaraz, M. (2002): Radioprotective effects *in vivo* of phenolics extracted from *Olea europaea* L. leaves against X-ray-induced chromosomal damage: comparative study versus several flavonoids and sulfur-containing compounds. J. Med. Food., **5**, 125-135.

Benhusein, G.M., Mutch, E., Aburawi, S. and Williams, F.M. (2010): Genotoxic effect induced by hydrogen peroxide in human hepatoma cells using comet assay. Libyan J. Med., (in press), (doi: 10.3402/ljm.v5i0.4637).

Bouallagui, Z., Han, J., Isoda, H. and Sayadi, S. (2011): Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. Food. Chem. Toxicol., **49**, 179-184.

Cumaoğlu, A., Rackova, L., Stefek, M., Kartal, M., Maechler, P. and Karasu, C. (2011): Effects of olive leaf polyphenols against

H_2O_2 toxicity in insulin secreting β-cells. Acta. Biochim. Pol., **58**, 45-50.

David, D., George, I.A. and Peter, J.V. (2007): Toxicology of the newer neonicotinoid insecticides: imidacloprid poisoning in a human. Clin. Toxicol., **45**, 485-486.

DeMicco, A., Cooper, K.R., Richardson, J.R. and White, L.A. (2010): Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos. Toxicol. Sci., **113**, 177-186.

Djelic, N. and Djelic, D. (2000): Evaluation of cytotoxic and genotoxic effects of permethrin using *in vitro* micronucleus test. Acta. Veterinaria-Beograd., **50**, 263-270.

Erel, O. (2004): A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin. Biochem., **37**, 277-285.

Erel, O. (2005): A new automated colorimetric method for measuring total oxidant status. Clin. Biochem., **38**, 1103-1111.

Falcioni, M.L., Nasuti, C., Bergamini, C., Fato, R., Lenaz, G. and Gabbianelli, R. (2010): The primary role of glutathione against nuclear DNA damage of striatum induced by permethrin in rats. Neuroscience, **168**, 2-10.

Fares, R., Bazzi, S., Baydoun, S.E. and Abdel-Massih, R.M. (2011): The antioxidant and anti-proliferative activity of the Lebanese *Olea europaea* extract. Plant. Foods. Hum. Nutr., **66**, 58-63.

Fojut, T.L. and Young, T.M. (2011): Desorption of pyrethroids from suspended solids. Environ. Toxicol. Chem., **30**, 1760-1766.

Gabbianelli, R., Nasuti, C., Falcioni, G. and Cantalamessa, F. (2004): Lymphocyte DNA damage in rats exposed to pyrethroids: effect of supplementation with Vitamins E and C. Toxicology, **203**, 17-26.

Gabbianelli, R., Falcioni, M.L., Nasuti, C., Cantalamessa, F., Imada, I. and Inoue, M. (2009): Effect of permethrin insecticide on rat polymorphonuclear neutrophils. Chem. Biol. Interact., **182**, 245-252.

Geyikoglu, F., Turkez, H. and Aslan, A. (2007): The protective roles of some lichen species on colloidal bismuth subcitrate genotoxicity. Toxicol. Ind. Health., **23**, 487-92.

Goulas, V., Exarchou, V., Troganis, A.N., Psomiadou, E., Fotsis, T., Briasoulis, E. and Gerothanassis, I.P. (2009): Phytochemicals in olive-leaf extracts and their antiproliferative activity against cancer and endothelial cells. Mol. Nutr. Food Res., **53**, 600-608.

Hu, F., Li, L., Wang, C., Zhang, Q., Zhang, X. and Zhao, M. (2010): Enantioselective induction of oxidative stress by permethrin in rat adrenal pheochromocytoma (PC12) cells. Environ. Toxicol. Chem., **29**, 683-690.

Institóris, L., Undeger, U., Siroki, O., Nehéz, M. and Dési, I. (1999): Comparison of detection sensitivity of immuno- and genotoxicological effects of subacute cypermethrin and permethrin exposure in rats. Toxicology, **137**, 47-55.

IPCS (International Program on Chemical Safety) (1985): Environmental health criteria 46 guidelines for the study of genetic effects in human populations. WHO, Geneva. pp.45-54.

Issam, C., Zohra, H., Monia, Z. and Hassen, B.C. (2011): Effects of dermal sub-chronic exposure of pubescent male rats to permethrin (PRMT) on the histological structures of genital tract, testosterone and lipoperoxidation. Exp. Toxicol. Pathol., **63**, 393-400.

Jemai, H., Bouaziz, M., Fki, I., El Feki, A. and Sayadi, S. (2008): Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chem. Biol. Interact., **176**, 88-98.

Jin, Y., Chen, R., Liu, W. and Fu, Z. (2010): Effect of endocrine disrupting chemicals on the transcription of genes related to the innate immune system in the early developmental stage of zebrafish (*Danio rerio*). Fish. Shellfish. Immunol., **28**, 854-861.

Kaeidi, A., Esmaeili-Mahani, S., Sheibani, V., Abbasnejad, M., Rasoulian, B., Hajializadeh, Z. and Afrazi, S. (2011): Olive (*Olea europaea* L.) leaf extract attenuates early diabetic neuropathic pain through prevention of high glucose-induced apoptosis: *In vitro* and *in vivo* studies. J. Ethnopharmacol., **136**, 188-196.

Kapoor, U., Srivastava, M.K., Bhardwaj, S. and Srivastava, L.P. (2010): Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL). J. Toxicol. Sci., **35**, 577-581.

Katsiki, M., Chondrogianni, N., Chinou, I., Rivett, A.J. and Gonos, E.S. (2007): The olive constituent oleuropein exhibits proteasome stimulatory properties *in vitro* and confers life span extension of human embryonic fibroblasts. Rejuvenation. Res., **10**, 157-172.

Khayyal, M.T., el-Ghazaly, M.A., Abdallah, D.M., Nassar, N.N., Okpanyi, S.N. and Kreuter, M.H. (2002): Blood pressure lowering effect of an olive leaf extract. (Olea europaea) in L-NAME induced hypertension in rats. Arzneitmittelforschung, **52**, 797-802.

Kusano, C. and Ferrari, B. (2008): Total antioxidant capacity: a biomarker in biomedical and nutritional studies. J. Cell Mol. Biol., **7**, 1-15.

Lee, O.H. and Lee, B.Y. (2010): Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. Bioresour. Technol., **101**, 3751-3754.

Mijatovic, S.A., Timotijevic, G.S., Miljkovic, D.M., Radovic, J.M., Maksimovic-Ivanic, D.D., Dekanski, D.P. and Stosic-Grujicic, S.D. (2011): Multiple antimelanoma potential of dry olive leaf extract. Int. J. Cancer, **128**, 1955-1965.

Nasuti, C., Gabbianelli, R., Falcioni, M.L., Di Stefano, A., Sozio, P. and Cantalamessa, F. (2007): Dopaminergic system modulation, behavioral changes, and oxidative stress after neonatal administration of pyrethroids. Toxicology, **229,** 194-205.

O'Brien, N.M., Carpenter, R., O'Grady, M.N. and Kerry, J.P. (2006): Modulatory effects of resveratrol, citroflavan-3-ol, and plant-derived extracts on oxidative stress in U937 cells. J. Med. Food, **9**, 187-195.

Omar, S.H. (2010): Oleuropein in olive and its pharmacological effects. Sci. Pharm., **78**, 133-154.

Poudyal, H., Campbell, F. and Brown, L. (2010): Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J. Nutr., **140**, 946-953.

Ramkumar, K.M., Sankar, L., Manjula, C., Krishnamurthi, K., Saravana, Devi. S., Chakrabarti, T., Kalaiselvi, K., Palanivel, M. and Rajaguru, P. (2010): Antigenotoxic potential of *Gymnema montanum* leaves on DNA damage in human peripheral blood hymphocytes and HL-60 cell line. Environ. Mol. Mutagen., **51**, 285-293.

Speranza, L., Franceschelli, S., Pesce, M., Reale, M., Menghini, L., Vinciguerra, I., De Lutiis, M.A., Felaco, M. and Grilli, A. (2010): Antiinflammatory effects in THP-1 cells treated with verbascoside. Phytother. Res., **24**, 1398-1404.

Srivastava, A., Srivastava M.K and Raizada R.B. (2006): Ninety-day toxicity and one-generation reproduction study in rats exposed to allethrin-based liquid mosquito repellent. J. Toxicol. Sci., **31**, 1-7.

Surrallés, J., Xamena, N., Creus, A., Catalán, J., Norppa, H. and Marcos, R. (1995): Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cul-

tures. Mutat. Res., **341**, 169-184.

Tisch, M., Schmezer, P., Faulde, M., Groh, A. and Maier, H. (2002): Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells. Eur. Arch. Otorhinolaryngol., **259**, 150-153.

Turkez, H. (2011): The role of ascorbic acid on titanium dioxide-induced genetic damage assessed by the comet assay and cytogenetic tests. Exp. Toxicol. Pathol., **63**, 453-457.

Undeğer, U and Başaran, N. (2005): Effects of pesticides on human peripheral lymphocytes *in vitro*: induction of DNA damage. Arch. Toxicol., **79**, 169-176.

Vadhana, M.S., Nasuti, C. and Gabbianelli, R. (2010): Purine bases oxidation and repair following permethrin insecticide treatment in rat heart cells. Cardiovas. Toxicol., **10**, 199-207.

Vijayalaxmi, Reiter, R.J., Leal, B.Z. and Meltz, M.L. (1996): Effect of melatonin on mitotic and proliferation indices, and sister chromatid exchange in human blood lymphocytes. Mutat. Res., **351**, 187-192.

Vontas, J.G., Small, G.J. and Hemingway, J. (2001): Glutathione S-transferases as antioxidant defence agents confer pyrethroid resistance in Nilaparvata lugens. Biochem. J., **357,** 65-72.

Wang, G.G., Lu, X.H., Li, W., Zhao, X. and Zhang, C. (2011): Protective effects of luteolin on diabetic nephropathy in STZ-induced diabetic rats. Evid. Based. Complement. Alternat. Med., 323171.

Zhang, S.Y., Ueyama, J., Ito, Y., Yanagiba, Y., Okamura, A., Kamijima, M. and Nakajima, T. (2008): Permethrin may induce adult male mouse reproductive toxicity due to cis isomer not trans isomer. Toxicology, **248**, 136-141.

Zrelli, H., Matsuoka, M., Kitazaki, S., Zarrouk, M. and Miyazaki, H. (2011): Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. Eur. J. Pharmacol., **660**, 275-282.