Olive leaf extract modulates permethrin induced genetic and oxidative damage in rats

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Abstract Permethrin is a common synthetic chemical, widely used as an insecticide in agriculture and other domestic applications. The previous reports indicated that permethrin is a highly toxic synthetic pyrethroid pesticide to human and environmental health. Therefore, the present experiment was undertaken to determine the effectiveness of olive leaf extract in modulating the permethrin induced genotoxic and oxidative damage in rats. The animals used were broadly divided into four (A, B, C and D) experimental groups. Group A rats served as control animals and received distilled water intraperitoneally ($n = 5$). Groups B and C rats received intraperitoneal injections of permethrin ($60 \text{ mg kg}^{-1} \text{ b.w}$) and olive leaf extract ($500 \text{ mg kg}^{-1} \text{ b.w}$), respectively. Group D rats received permethrin ($60 \text{ mg kg}^{-1} \text{ b.w}$) plus olive leaf extract ($500 \text{ mg kg}^{-1} \text{ b.w}$). Rats were orally administered their respective feed daily for 21 days. At the end of the experiment rats were anesthetized and serum

and bone marrow cell samples were obtained. Genotoxic damage was assessed by micronucleus and chromosomal aberration assays. Total antioxidant capacity and total oxidant status were also measured in serum samples to assess oxidative status. Treatment of Group B with permethrin resulted in genotoxic damage and increased total oxidant status levels. Permethrin treatment also significantly decreased ($P < 0.05$) total antioxidant capacity level when compared to Group A rats. Group C rats showed significant increases ($P < 0.05$) in total antioxidant capacity level and no alterations in cytogenetic parameters. Moreover, simultaneous treatments with olive leaf extract significantly modulated the toxic effects of permethrin in Group D rats. It can be concluded that olive leaf extract has beneficial influences and could be able to antagonize permethrin toxicity. As a result, this investigation clearly revealed the protective role of olive leaf extract against the genetic and oxidative damage by permethrin in vivo for the first time.

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Introduction

Olea europea L. leaves contain polyphenolic compounds (hydroxytyrosol, oleuropein, secoiridoids, flavonoids and triterpenes) that may protect in tissue

cells against oxidative stress (Kranz et al. 2010; Fares et al. 2011; Bouallagui et al. 2011). European and Mediterranean countries have been used in the human diet as extract, herbal tea, and powder. Since these leaves contain many potentially bioactive compounds, they may have antioxidant properties (El and Karakaya 2009; Moussaoui et al. 2010). At the same time olive leaves have been heavily exploited for the prevention or the treatment of hypertension, carcinogenesis, diabetes, atherosclerosis, gingivitis and many other traditional therapeutic uses (Han et al. 2009; Haloui et al. 2010; Bouallagui et al. 2011). Poudyal et al. (2010) reported that olive leaf extract (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. In addition, OLE possessed gastro-protective activity against cold restraint stress-induced gastric lesions in rats, possibly related to its antioxidant properties (Dekanski et al. 2009). Interestingly Bao et al. (2007) found that OLE has anti-HIV activity by blocking the HIV virus entry to host cells.

Permethrin ($C_{21}H_{10}Cl_2O_3$), (PM) entered use in the 1970s as an insecticide in a wide range of applications, including agriculture, horticultural, and forestry (Turner et al. 2010). The previous reports showed that PM was a highly toxic synthetic pyrethroid pesticide widely used in agriculture and vector control programs. About 60% of the PM produced is used on cotton plants. Other crops to which PM is applied are maize, soya beans, coffee, tobacco, rape seed oil, wheat, barley, alfalfa, vegetables and fruit (WHO 1990). It was found that PM is highly toxic to fish; but it is less toxic to guppies (Başer et al. 2003). Likewise, Sutton et al. (2007) reported that 96.9% of exposed cats developed clinical effects, 87.8% developed increased muscular activity and 10.5% of cases resulted in fatalities. PM was 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).

So far, antioxidants have attracted much interest with respect to their protective effect against damage by free radical that may be the cause for many diseases including cancer (Shon et al. 2004). Since the complete avoidance of exposure to PM is very difficult, chemoprevention is an attractive strategy for protecting humans and animals from the risk of cancer caused

by exposure to this insecticide. In fact, recent studies focused on exploring protective agents, such as vitamins C and E, coenzyme Q(10) and glutathione, against PM toxicity (Vontas et al. 2001; Gabbianelli 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 study we evaluated the effects of OLE against PM-induced DNA damages for improving its therapeutic gain. Firstly, important oxidative parameters, TAS (Total antioxidant capacity) and TOS (Total oxidant status) were used to monitor the development and extent of damage due to oxidative stress in rat blood serum (Turkez et al. 2012). In addition, chromosomal aberrations (CA) and micronucleus (MN) test providing sensitive and rapid monitoring of induced genetic damage as primary DNA damage were performed on bone marrow cells (de Souza et al. 2006).

Materials and methods

Plant materials and extracts

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 dried in the shade and crushed. After crushing, 100 g of powdered leaves was extracted. The extract was manufactured from the dried leaves of *O. europaea* applying ethyl acetate extraction procedure previously reported by Turkez and Togar (2011).

Animals and chemicals

Experiment was carried out on male Sprague–Dawley rats, 8-weeks old, weighing 180–200 g. The animals were kept on a 12-h light–dark cycle and allowed free access to food and water. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). PM (Cas No 52645-53-1; $C_{21}H_{20}Cl_2O_3$) was obtained from Riedel–de Haen® company (Germany). All other chemicals were purchased from Sigma® (USA).

Experimental design

The animals were randomly divided into equal four groups ($n = 5$): group A: rats were given an equivalent

of distilled water, group B: Rats received OLE (500 mg kg⁻¹), group C: rats received PM (60 mg kg⁻¹) and group D: rats received OLE plus PM for 21 days intraperitoneally. The doses were selected according to literature data (Van Haaren et al. 2000; Hussain et al. 2010). After the treatments of PM, OLE and PM plus OLE, the animals were anesthetized with ether.

Biochemical and cytogenetic studies

Blood samples were collected into serum separator tubes (Microtainer; Becton–Dickinson, Franklin Lakes, NJ, USA). The samples were allowed to stand (75–90 min) and centrifuged (860 g for 20 min) for obtaining serum samples. The TAC and TOS levels were determined spectrophotometrically on serum samples using commercially available kits (Assay Diagnostics, Turkey) (Erel 2004; Erel 2005).

Bone marrow cell preparations for the analysis of CAs (gaps and breaks) were produced by the colchicine–hypotonic citrate technique. Potassium chloride (0.075 M) was used in this technique. Colchicine (0.1%, 1 ml⁻¹ 100 g body wt) was injected i.p. 90 min before killing the animals. Animals were killed and then bone marrow cells were flushed from the femora with 0.075 M potassium chloride. Slides were prepared by an air drying procedure and stained with 5% Giemsa stain (Assayed et al. 2010). Criteria to classify the different types of aberrations were in accordance with the recommendation of Environmental Health Criteria (EHC) 46 for environmental monitoring of human populations (IPCS 1985). Structural and numerical chromosomal aberrations (CA) were scored in 100 metaphases per animal (a total of 500 metaphases for each group). For MN analysis, bone marrow was isolated from the other femur bone with the help of syringes and homogenized with fetal bovine serum (FBS). The slides were coded, fixed with methanol and stained with Giemsa solution. Two thousand polychromatic erythrocytes (PCE) from each animal were scored for MN presence (Sekeroglu et al. 2011). Slides were scored in duplicates at a magnification of 1,000× using a light microscope by one observer (B. Tögar).

Statistical analysis

The statistical analysis of experimental values in the CA, MN and TAC- TOS analysis was performed by oneway analysis of variance (ANOVA) and Fisher's

LSD 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

Treatment with PM resulted in a significant ($P < 0.05$) decrease in serum TAC level, while causing a significant increase in TOS level. Treatment with OLE alone did not affect TOS level, but led to increases of TAC level. However, in its combination with PM, the OLE significantly ($P < 0.05$) alleviated the distortion in TAC and TOS levels (Table 1). In addition, our results showed that OLE did not alter CA and MN frequencies in bone marrow cells. PM significantly increased the rates of CA and MN formation in bone marrow cells as compared with controls. When the OLE and PM were given together to animals, OLE reduced the number of PM-induced CA and MN formation but it did not completely revert the PM caused genetic damages (Table 2).

Discussion

Our results clearly indicated the PM induced genotoxic and oxidative damage in rats in vivo. Similarly to

Table 1 The TAC and TOS levels in rats serum samples simultaneously exposed PM and OLE

Treatments	Values	
	TAC (mmol Trolox Equiv l ⁻¹)	TOS (μmol H ₂ O ₂ Equiv l ⁻¹)
Group A (control)	4.1 ± 0.7 ^c	5.7 ± 0.9 ^a
Group B (OLE; 500 mg kg ⁻¹)	5.9 ± 0.6 ^d	5.5 ± 1.3 ^a
Group C (PM; 60 mg kg ⁻¹)	2.8 ± 0.5 ^a	7.8 ± 1.4 ^c
Group D (OLE plus PM)	3.5 ± 0.5 ^b	6.2 ± 1.1 ^b

(Values are means ± SD of five experiments. Means shown by the same letter are not significantly different from each other at a level of 5%, using Duncan's test. For example, the means shown by the letter b is different from a or c. In other words, the means having different letters are different from each other)

PM permethrin, OLE olive leaf extract, TAC total antioxidant capacity, TOS total oxidant status

Table 2 Frequency of MN and CA in bone marrow cells simultaneously exposed to PM and OLE

Treatments	Values	
	Number of MNPCE per animal	Number of CAs per animal
Group A (control)	0.46 ± 0.22 ^a	1.82 ± 0.46 ^a
Group B (OLE; 500 mg kg ⁻¹)	0.48 ± 0.25 ^a	1.76 ± 0.55 ^a
Group C (PM; 60 mg kg ⁻¹)	3.85 ± 1.14 ^c	4.47 ± 0.87 ^c
Group D (OLE plus PM)	1.54 ± 0.73 ^b	2.66 ± 0.81 ^b

(Values are means ± SD of five experiments. Means shown by the same letter are not significantly different from each other at a level of 5%, using Duncan's test. For example, the means shown by the letter b is different from a or c. In other words, the means having the different letters are different from each other)

MNPCE micronucleated polychromatic erythrocytes, *CA* chromosomal aberrations, *chromatid/chromosome gaps* chromatid/chromosome breaks and centric fusions or fragments were considered as same in CA assay

our findings, 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 another report, PM significantly increased the DNA damage in a concentration-dependent manner in healthy human lymphocytes (Undeger and Basaran 2005). 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. Moreover, findings provided evidence for the potential carcinogenicity of PM to human nasal mucosal cells. PM gave mostly negative results, although it increased the MN frequency in human blood cultures (Surralles 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 rats. Because, the PM administration (group C) caused increases of TOS level and decrease of TAC level as compared to control group (group A). In accordance with our findings, recent investigations clearly indicated that PM-induced oxidative stress leads to biochemical and

functional changes in organisms (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 was 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 proliferation at low micro molar concentrations (Goulas et al. 2009). Thus, OLE probably modulated the PM-induced genetic and oxidative damage by preventing free radical generation or by stimulating components of the antioxidant defense system. In fact, OLE was reported to have free oxygen radicals and lipoperoxyl radicals scavenging capacity and, anti-clastogenic activity due to its polyphenolic contents, mainly catechol groups (rutin, oleuropein, hydroxytyrosol, verbascoside, luteolin) (Benavente-García et al. 2002).

In conclusion the authors recommend the consumption of plenty of OLE within the food especially for humans that work in agriculture or are overtly exposed to various pesticides or consume pesticide-treated fruits and vegetables in order to protect themselves and their offspring against any expected harm. Bestowed with strong antioxidant and genoprotective potentials, the nutraceutical value of olive leaves make them ideal candidates to protect against pesticide-induced mutagenicity or carcinogenicity.

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