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Olive phenolic compounds attenuate deltamethrin-induced liver and kidney toxicity through regulating oxidative stress, inflammation and apoptosis

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ABSTRACT

The purpose of this study was to evaluate the protective effect of ethanolic olive fruit extract (OFE) and its phenolic compound, oleuropein (OLE), against hepato-renal toxicity induced by deltamethrin (DEM), a synthetic pyrethroid, in Wistar rats. The kidney and liver tissues were collected after 30 days of treatment for subsequent investigation. Rats that were given DEM had a highly significant elevation in the serum biomarkers as well as hepatic and renal levels of lipid peroxidation (MDA). Additionally, a significant reduction in the total antioxidant capacity (ABTS+), superoxide dismutase (SOD) and catalase (CAT) activities was noted. This toxic effect was confirmed by histological studies and the expression levels of inflammatory (cox-2) and apoptotic genes (bcl-2 and p53). The findings for the OFE and OLE-treated groups highlighted the efficacy of olive fruit phenolic compounds as hepatic and renal-protectant in DEM-induced hepato-renal toxicity through improving the oxidative status as well as suppressing inflammation and apoptosis. Therefore, they may be used as protective natural compounds against DEM-induced hepato-renal toxicity.

1. Introduction

Medicinal plants play an important role in protecting human health against harmful chemicals including pesticides that may cause oxidative stress, leading to the generation of free radicals and the alteration in antioxidant enzyme systems (Banerjee et al., 1999). Lately, several studies have indicated that fruits and leaves of the olive plant (*Olea europaea*) are rich in polyphenols, thus exhibiting a range of beneficial effects, including anti-inflammatory, antioxidant and anti-carcinogenic properties (Omar, 2010).

Human and especially farmers are in continuous contact to insecticides, extensively used worldwide, either directly or indirectly (Abdel-Daim and El-Ghoneimy, 2015). Consequently, the intensive use of pesticides increases potential human health problems, including acute, subacute, as well as chronic poisonings and thus, are main reasons of interest (Abdel-Daim and Halawa, 2014). Kidney and liver are among the most common chemical-affected tissues. Indeed, these toxicants can cause severe renal damage including inflammation, tubular cell toxicity, crystal nephropathy (Schetz et al., 2005; Zager, 1997) and nephrotoxicity in rat models (Behling et al., 2006). They also cause liver tissue alteration through inducing liver fibrosis and cirrhosis in experimental animals (Al-Attar and Shawush, 2015). Among these pesticides, several studies reported that deltamethrin (DEM) is a synthetic pyrethroid that can elicit neurotoxicity, and lead to apoptosis (Khalatbary et al., 2015). Recent studies investigated the oxidative damage of DEM in experimental animals, inducing apoptosis in rat brain (Ogaly et al., 2015), acute and subacute toxicities in rat liver and kidney (Abdel-Daim et al., 2014, 2013), as well as subacute injury in liver, kidney and gill tissues in freshwater fish Nile tilapia (Abdel-Daim et al., 2015; Abdelkhalek et al., 2015). The accumulation of DEM in body systems enhances the reactive oxygen species (ROS) production. The deleterious effects of ROS accumulation introduced damage to all macromolecules including proteins, nucleic acids and, lipids. Under normal circumstances, the body is endowed with efficient antioxidant defense systems to reverse the menace of oxidative stress (Galal et al., 2014).

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On this line, the protective effect of some bioactive compounds against DEM-induced toxicities has been investigated in experimental animals (Abdel-Daim et al., 2016; Abdou and Abdel-Daim, 2014). Moreover, Khalatbary et al. (2015) demonstrated the anti-apoptotic effect of oleuropein pretreatment against DEM-induced damage in Cerebellar Purkinje Neurons. Ogaly et al. (2015) investigated the influence of green tea extract on DEM-induced oxidative damage in rat brain. In addition, Gündüz et al. (2015) showed that glutamine, an essential amino acid, provides effective protection against DEM-induced hepatotoxicity.

It is well established that olive compounds such as oleuropein and hydroxytyrosol, play a key role against oxidative stress in mammalian cells (Cerig et al., 2016; Martínez-Martos et al., 2014). Indeed, oleuropein, which the majority of the polyphenols found in table olives are products of its hydrolysis, has high antioxidant activity *in vitro*, comparable to ascorbic acid and more potent than trolox, a hydrosoluble analog of tocopherol (Speroni et al., 1998). However, the effect of olive compounds on DEM-induced damage in kidney and liver tissues is still poorly studied. This study aimed to investigate the *in vitro* effects of DEM on oxidative stress parameters as well as liver and kidney tissues and then, to evaluate the protective effects of olive fruit extract (OFE) and oleuropein (OLE) against renal and hepatic alterations associated with oxidative stress.

2. Materials and methods

2.1. Plant material and preparation of extract

Olea europeae fruits from Jerboui cultivar were collected in the area of Beja prefecture. Olive fruit extract was prepared in our laboratory by soaking a homogeneous paste of 200 g of olives in 350 mL of ethanol (70%) overnight, under shaking at room temperature. The resulted ethanolic extract was filtered and the lipid fraction was removed using hexane. The resulting extract was then freeze-dried and stored for further analyses. The extraction yield was in the order of 10%.

Oleuropein used in the current study was purified from dried olive leaves according to Hadrich et al. (2016). 100 g of dried olive leaves were soaked in 500 mL distilled water. The mixture was kept under agitation at room temperature overnight and then filtered. The resulting aqueous phase was extracted thrice with equal volume of ethyl acetate and the organic phase was dried under vacuum. The residue was then chromatographed at low pressure on silica gel using a mixture of methylene chloride and methanol. Finally, the purified fraction was freeze-dried and stored until use. The purity level of oleuropein was 97% with a purification yield of 5.7% of dry olive leaves.

2.2. Compositional characterization of OFE

A high performance liquid chromatography analysis was performed to identify and quantify the major phenolic compounds of the OFE. The phenolic profile of OFE was obtained following the method of Souilem et al. (2014) using an Agilent series 1260 HPLC-DAD instrument (Agilent Technologies, Waldbronn, Germany).

Detection was performed using a diode array detector (DAD), and the chromatograms were recorded at $\lambda = 254$ nm for oleuropein, and at 330 nm for flavonoids (Luteolin glucoside, apigenin-7-glucoside and verbascoside). Quantification was performed by external calibration with standards.

The purity level of OLE was determined using LC–MS/MS (Agilent 1100 LC, Germany) equipment according to Bouallagui et al. (2011).

2.3. Cell culture

Human Embryonic Kidney (HEK-293) and Hepatic (HepG-2) cells were grown in 10% (v/v) fetal bovine serum (FBS) and 1% PS-supplemented DMEM medium (Life Technologies, UK). Cells were incubated at 37 °C in a modified atmosphere of 5% CO_2 and 95% air until confluence.

2.4. Colorimetric cytotoxic assay

Cytotoxicity experiments were performed to determine the IC50 values defined as the doses of OFE and OLE required for the 50% inhibition of cell growth. The viability was evaluated by MTT assay (Mosmann, 1983). The cells were seeded in 96-well plates at a density of 3×10^4 live cells/mL and treated with different concentrations of OFE (0–1800 μg/mL) and purified OLE (0–1000 μg/mL) for different incubation times (24, 48 and 72 h). 100 μL new medium and 10 μL MTT solution (5 mg/mL) was then added to each well and incubated for 4 h at 37 °C. Finally, the formazan crystal formed was dissolved by adding 100 μL of SDS and the absorbance was detected at 570 nm using a microplatereader (Thermo Scientific Varioskan Flash). Relative cell viability of the different treatments was calculated as a percentage of the control cells viability.

2.5. Animals and experimental protocol

Twenty-four Wistar male rats, about 200 ± 10 g body weight (BW), were purchased from the Central Pharmacy (SIPHAT, Tunisia). They were maintained at 22 ± 4 °C with light/dark periods of 12 h and a minimum relative humidity of 40%. Animals had a free access to water and commercial diet (SNA, Sfax, Tunisia).

After the one-week adaptation period, animals were randomly divided into four groups ($n = 6$) for the experimental procedure. Treatment was then carried out as follows: Group 1 (G1), which served as the control group. Group 2 (G2), was given a suspension of DEM (15 mg/kg BW). The selected dose of DEM was based on previous studies where 1/10 of the LD_{50} induced biochemical alterations in rats without leading to morbidity (Abdel-Daim et al., 2013). Group 3 (G3) and Group 4 (G4) were treated with OFE (200 mg/kg BW) and OLE (50 mg/kg BW), respectively 1 h before DEM administration at the same dose of group 2. All treatments were given orally using stomach gavage, and continued for 30 days.

2.6. Sample extraction

After 30 days from the beginning of the treatment, experimental animals were precisely weighed, anesthetized with ether and sacrificed. Blood serum was withdrawn, in heparinised tubes, from the brachial artery of rats and centrifuged (2200 *g*, 15 min, 4 °C). Kidneys and livers were carefully dissected out and weighed. All samples were stored at - 80 °C for later biochemical and histological analysis.

2.7. Biochemical analysis

Serum kidney and liver biomarkers (urea, creatinine, alanine aminotransferase: ALT and Aspartate aminotransferase: AST) activities, serum lipid levels of high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were measured using an automatic biochemistry analyzer (Vitalab FlexorE, USA) at the biochemical laboratory (Hedi Chaker University Hospital, Sfax- Tunisia).