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Original article

EFFECT OF OLIVE OIL ON OXIDATIVE AND DNA DAMAGE INDUCED BY IMIDACLOPRID

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Summary

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The current study aimed to evaluate the effect of olive oil (OLO) on the genotoxicity of imidacloprid (IMI). Rats were divided into four groups: control group, IMI group that was exposed to 22.5 mg/kg b.w. IMI for 4, 8, and 12 weeks; OLO before IMI group – dosed orally with OLO at a dose of 10 mL/kg body weight for two weeks before IMI exposure of 4, 8, and 12 weeks. The OLO after IMI group was treated with OLO in a dose of 10 mL /kg body weight for two weeks after IMI exposure of 4, 8, and 12 weeks. Malondialdehyde (MDA) and glutathione peroxidase (GPx) levels were measured in blood serum. The bone marrow from the femur was collected for the comet assay. The results revealed that exposure to IMI induced DNA damage, which was associated with increased blood MDA level and decreased GPx activity. However, treatment with OLO resulted in decreasing both DNA damage and MDA level while increasing GPx activity. On the other hand, pre-treatment with OLO was effective in decreasing the DNA damaging effect of IMI.

Key words: comet assay, imidacloprid, olive oil, oxidative DNA damage, oxidative stress

INTRODUCTION

Agricultural uses of pesticides can result in contamination of the environment by toxic compounds (Batikian *et al.*, 2019). Neonicotinoids are used as agrochemicals to manage pests in over 120 countries (Pisa *et al.*, 2015). Imiclopramid – IMI; [1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine] is the most commonly used insecticide of the neonicotinoid group (Tomizawa & Yamamoto, 1993). Neurotoxicity of neonicotinoids in insects due to irreversible binding to nicotinic acetylcholine receptors, leads to paralysis and death (Tomizawa & Casida, 2005). Several nutritional studies have discussed the ability of dietary components to enhance biological functions (Das *et al.*, 2012). Phytochemicals in fruits and vegetables have the potential to strengthen health and/or

prevent diseases (Ganann *et al.*, 2014). Olive fruits and oil contain different types of phenols such as simple phenol (hydroxytyrosol, tyrosol), polyphenols (oleuropein glucoside); and other constituents: secoiridoids, the dialdehydic form of oleuropein (Rahmani *et al.*, 2014). These phenolic compounds have strong antioxidant and radical scavenging effects (Masella *et al.*, 2004) and also a protective effect against cancer at high intake levels (Franceschi *et al.*, 1999). Several studies (*in vivo* and *in vitro*) have found that hydroxytyrosol and oleuropein in olive oil are strong antioxidants due to their ability to inhibit lipid peroxidation and scavenge free radicals (Léger *et al.*, 2000). The toxicity of pesticides entails excessive production of reactive oxygen species (ROS) (Kale *et al.*, 1999) that increase the lipid peroxidation in cell membranes (Jacobsen-Pereira *et al.*, 2018) resulting in damage to mammalian cells. Such damage, if not repaired, may lead to DNA damage and mutation in somatic cells (Saran *et al.*, 2004).

Therefore, the present study aimed to evaluate the protective and therapeutic effects of olive oil against the genotoxic effect and oxidative stress induced by IMI in male Sprague Dawley rats.

MATERIALS AND METHODS

Chemicals

Chemicals used in the present study comprised: a commercial formulation of imiclopramid (Suzhou World Best Agro Biochemical Company, China) as a wettable powder containing 25% (w/w) IMI as active ingredient; refined OLO blended with extra virgin OLO (Rafael Salgado, Madrid, Spain); sodium chloride, sodium lauryl sulphate, disodium ethylenediaminetetraacetic acid (El Nasr Pharmaceutical Chemicals Co., Egypt); normal agarose, low melting point agarose and Tris base (Winlab Laboratory Chemicals Co., U.K.); dimethyl sulfoxide, Triton X-100 (Oxford Laboratory Chemicals, Mumbai, India), sodium hydroxide (United Chemical Laboratory CO., Egypt); ethidium bromide stain (FUJIFILM Wako Pure Chemical Corporation, Japan).

Animals

A total of 120 male Sprague Dawley rats (100–150 g body weight; 10–12 weeks of age) were purchased from the Animal House, Faculty of Medicine, Assiut University, Egypt. Rats were maintained in plastic cages and given commercial pellets and clean water *ad libitum*. Animals were acclimated to the laboratory conditions two weeks before the experiment. All animals were housed under a suitable temperature and lighting cycle of 12 hours light/12 hoursdark. All protocols were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt (April 2018).

Experimental design

Animals were divided into four groups 30 animals each:

- Group I: Control group – rats were administered 1 mL/100 g distilled water orally using a stomach tube day by day for 4, 8, and 12 weeks;
- Group II: IMI group – rats were administered imiclopramid dissolved in distilled water at 22.5 mg/kg body weight by oral gavage, day by day for 4, 8, and 12 weeks. This dose represents 1/20 of the oral LD_{50} (450 mg/kg body weight) according to Meister (1994).
- Group III: OLO before IMI group: rats were divided into three subgroups (A, B, and C) and were dosed

orally with OLO at a dose of 10 mL/kg body weight according to Antunes &Takahashi (1999), day by day for two weeks before IMI exposure of 4, 8, and 12 weeks, respectively.

- Group IV: OLO after IMI group: rats were divided into three subgroups (A, B, and C) and received OLO at a dose of 10 mL/kg body weight orally, day by day for two weeks after IMI exposure of 4, 8, and 12 weeks, respectively. The dose volume was 1 mL/100 g body weight for all exposed animals.

Collection of samples

Ten animals from each group were sacrificed for samples collection after 4, 8, 12 weeks in groups I and II. In groups III and IV, samples were collected after 6, 10, 14 weeks. Blood samples were collected from the descending aorta in vacutainer tubes without anticoagulant to obtain serum after centrifugation at 3,000 rpm for 10 minutes for estimation of MDA and GPx. Bone marrow samples were collected from the femoral bones, which were dissected out and the bone marrow was flushed for the comet assay.

Comet assay

DNA damage in bone marrow was detected using comet assay (single cell gel electrophoresis) according to the method of Sasaki *et al.* (1997). Briefly, bone marrow samples were flushed in the chilled buffer then centrifuged at 3,000 rpm for 15 min at 0 °C. Fully frosted slides were layered twice with 100 μL of 1% normal agarose. Seventy-five μL of nuclear suspension (supernatant) was mixed with 75 μL of 2% agarose (low melting point agarose) at 45 °C and the mixture was layered on the fully frosted slide and covered with another slide. Finally, 100 μL of 1% agarose was quickly layered on the surface and covered with another slide, and allowed to gel. The slides were placed immediately into a chilled lysing solution and kept at 4 °C in the dark for 1–24 hours. The slides were placed on a horizontal gel electrophoresis platform (Cleaver Scientific Ltd, U.K.) and covered with a chilled alkaline solution in the dark at 0 °C for 20 min (unwinding period) then electrophoresis was conducted at 0 °C in the dark for 20 min at 25 V and 300 mA. The slides were rinsed with Tris buffer for 7 min. The slides were kept in ethanol for 5 min then allowed to dry at room temperature, and stained with 50 μL ethidium bromide just before the microscopical examination. The nuclei on the slides were examined using a fluorescence microscope (Olympus BX-43, Japan) equipped with a green filter. At least 150 cells per sample were analysed using the Comet Assay Software Project (CASP) (the University of Wroclaw, Institute of Theoretical Physics). Tail parameters including tail length (TL), % DNA in the tail, tail moment (TM), and olive tail moment (OTM) were calculated.

Estimation of serum MDA level and GPx activity

MDA level was measured at 534 nm according to the method of Ohkawa *et al.* (1979). GPx activity was determined at 340 nm according to the method of Paglia & Valentine (1967) using commercial kits (Biodiagnostic, Egypt) and UV-2100 spectrophotometer (Unico, USA).

Statistical analysis

The data were expressed as mean ± SEM. The results were analysed statistically using a one-way analysis of vari-

ance (ANOVA) followed by Tukey multiple comparison *post hoc* test. These analyses were carried out using the SPSS program for Windows, v.16.0 (Borenstein *et al.,* 1997). Differences between and among the groups were considered significant at P ≤ 0.05.

RESULTS

DNA damage parameters

The results of comet assay in rats exposed to IMI and those exposed to OLO before and IMI exposure are presented in Tables 1–4 and Fig. 1.

Tail length (TL). TL showed an insignificant change in all groups by the 4th week. By the 8th week a significant increase in TL was recorded in IMI exposed group (P<0.01), OLO before IMI group (P<0.05), and OLO after IMI group (P<0.01) when compared with the control group. OLO before IMI group and OLO after IMI group however showed a significant decrease (P<0.01) vs IMI exposed group. On the other hand, by the 12th week, a significant increase (P<0.01) was observed in IMI group and OLO after IMI group vs controls. A significant decrease (P<0.01) was observed in OLO before IMI group and OLO after IMI group in comparison with IMI group (Table 1).

Tail DNA %. Tail DNA % showed an insignificant change in all groups by the 4th week while by the 8th week there was a significant increase (P<0.01) in IMI expsed group and OLO after IMI group compared with the control group. There was a significant (P<0.01) decrease in

Table 1. Comet tail length (µm) indicating the effect of olive oil administered pre- and post imidacloprid exposure. Data are expressed as mean±SEM (n=10 rats/group)

Groups	Time of exposure (weeks)		
	4	8	12
G1 (control)	3.000 ± 0.129	3.384 ± 0.138	3.806 ± 0.161
G2 (imiclopramid)	3.208 ± 0.172	7.971 ± 0.505*	9.711 ± 0.829*
G3 (olive oil before imiclopramid)	3.095 ± 0.130	5.082 ± 0.299*#	5.373 ± 0.358#
G4 (olive oil after imiclopramid)	3.168 ± 0.100	6.026 ± 0.323*#	6.304 ± 0.557*#

Different symbols within columns indicate significant changes (P≤0.05) between groups: vs the control group G1 (*); vs G2 (#).

Table 2. Tail DNA % indicating the effect of olive oil administered pre- and post imidacloprid exposure. Data are expressed as mean±SEM (n=10 rats/group)

Groups	Time of exposure (weeks)		
	4	8	12
G1 (control)	2.026 ± 0.134	1.852 ± 0.063	1.874 ± 0.083
G2 (imiclopramid)	2.263 ± 0.148	3.726 ± 0.334*	6.124 ± 0.798*
G3 (olive oil before imiclopramid)	2.132 ± 0.188	2.150 ± 0.184#$	2.567 ± 0.332#
G4 (olive oil after imiclopramid)	2.146 ± 0.150	3.349 ± 0.250*$	3.568 ± 0.712#

Different symbols within columns indicate significant changes (P≤0.05) between groups: vs the control group G1 (*); vs G2 (#); between G3 and G4 ($).

Table 3. Tail moment indicating the effect of olive oil administered pre- and post imidacloprid exposure. Data are expressed as mean±SEM (n=10 rats/group)

Groups	Time of exposure (weeks)		
	4	8	12
G1 (control)	0.078 ± 0.006	0.116 ± 0.009	0.166 ± 0.013
G2 (imiclopramid)	0.094 ± 0.010	0.472 ± 0.075*	1.00 ± 0.150*
G3 (olive oil before imiclopramid)	0.080 ± 0.008	0.156 ± 0.020#	0.447 ± 0.054#
G4 (olive oil after imiclopramid)	0.082 ± 0.006	0.277 ± 0.028*#	0.794 ± 0.239*

Different symbols within columns indicate significant changes (P≤0.05) between groups: vs the control group G1 (*); vs G2 (#).

Table 4. Olive tail moment indicating the effect of olive oil administered pre- and post imidacloprid exposure. Data are expressed as mean±SEM (n=10 rats/group)

Groups	Time of exposure (weeks)		
	4	8	12
G1 (control)	0.698 ± 0.045	0.688 ± 0.027	0.698 ± 0.030
G2 (imiclopramid)	1.024 ± 0.189	1.573 ± 0.136*	2.110 ± 0.243*
G3 (olive oil before imiclopramid)	0.752 ± 0.064	0.922 ± 0.073#	1.116 ± 0.124#
G4 (olive oil after imiclopramid)	0.820 ± 0.057	1.122 ± 0.077*#	1.356 ± 0.238*#

Different symbols within columns indicate significant changes (P≤0.05) between groups: vs the control group G1 (*); vs G2 (#).

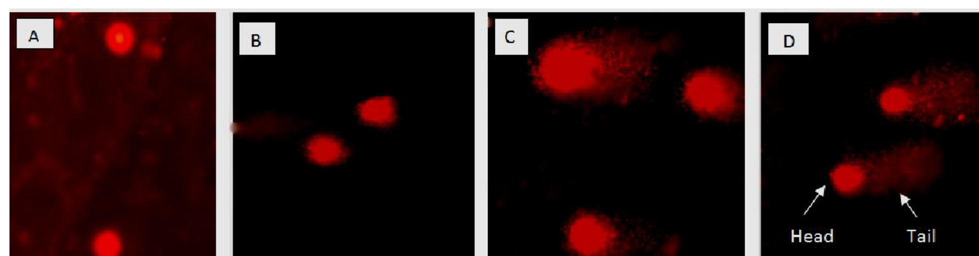

Fig. 1. Comet assay (single cell gel electrophoresis) showing control cells without DNA damage (**A**) and different degrees of DNA damage after exposure to imiclopramid for 4 (**B**), 8 (**C**) and 12 (**D**) weeks.

tail DNA % in OLO before IMI group compared with IMI exposed group and OLO after IMI group. In the 12th week, it showed a significant increase (P<0.01) in IMI group compared with the control group, and a significant decrease (P<0.01) in OLO before IMI group and OLO after IMI group compared with the group treated with IMI (Table 2).

Tail moment (TM) and olive tail moment (OTM). TM showed no significant change in all groups by the 4th week. It increased statistically significantly by the 8th week in IMI group (P<0.01) and OLO

after IMI group (P<0.05) compared with control animals. TM showed a significant reduction (P<0.01) in OLO before IMI group and OLO after IMI group vs the IMI group. By the 12th week, it showed a significant increase in IMI exposed group (P<0.01) and OLO after IMI group (P<0.05) compared with the control group, and a significant decrease in OLO before IMI group (P<0.05) compared to IMI group (Table 3). OTM showed an insignificant change in all groups by the 4th week. By the 8th week, it was significantly higher in IMI exposed group (P<0.01) and OLO after IMI group (P<0.05) compared with the controls whereas a significant decrease was recorded in OLO before IMI group (P<0.01) and OLO after IMI group (P<0.05) vs IMI group. By the 12th week, OTM increased considerably in IMI-treated group (P<0.01) and the group that received OLO after IMI (P<0.05) compared to untreated controls. There was a significant decrease in OTM in the OLO before IMI group (P<0.01) and OLO after IMI group (P<0.05) in comparison with IMI group (Table 4).

Blood oxidative parameters

Serum MDA level. The level of MDA showed a significant increase (P<0.05) by the 4th week in IMI group and OLO before IMI group when compared with controls. By the 8th week, a significant increase (P<0.01) was recorded in IMI group, OLO before IMI group and OLO after IMI group vs the control group, while a significant decrease occurred in OLO before IMI group (P<0.05) and OLO after IMI group (P<0.01) in comparison with IMI exposed group. By the 12th week, MDA was significantly increased (P<0.01) in all treated groups vs control group, and significantly lower (P<0.01) in OLO before IMI group, and OLO after IMI group compared with IMI exposed group (Fig. 2).

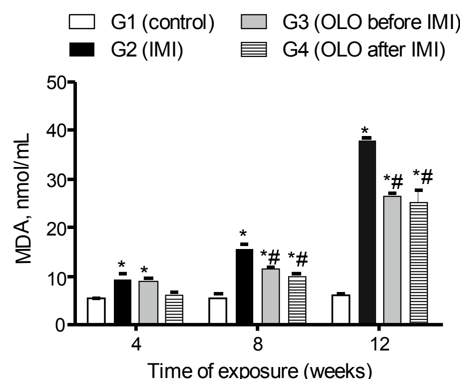

Fig. 2. Serum malondialdehyde level (mean ± SEM, nmol/mL) in control group (G1), imiclopramid group (G2), olive oil before imiclopramid group (G3) and olive oil after imiclopramid group (G4). Different symbols indicate significant changes between groups at P≤0.05 vs control group G1 (*); vs G2 (#).

Serum GPx activity. GPx activity showed a significant decrease (P<0.01) by the 4th week in IMI group, OLO before IMI group and OLO after IMI group compared with the control group. Serum activity of this enzyme was considerably elevated (P<0.01) in OLO before IMI group and OLO after IMI group compared with IMI group. By the 8th week, GPx activity significantly decreased (P<0.01) in IMI group, OLO before IMI group and OLO after IMI group vs controls. It remained significantly higher in OLO before IMI group (P<0.01) and OLO after IMI group (P<0.05) compared with IMI group. By the 12th week, a significant reduction (P<0.01) was detected in GPx activity in IMI group and OLO after IMI group vs control group, and a significant increase (P<0.01) – in OLO before IMI group and OLO after IMI group compared with IMI group. Also,

GPx activity in OLO before IMI group was significantly higher (P<0.01) than that in OLO after IMI group (Fig. 3).

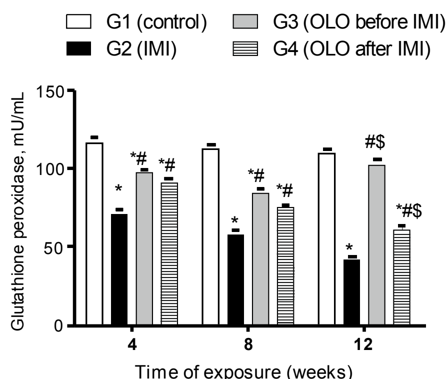

Fig. 3. Serum glutathione peroxidase activity (mean ± SEM, mU/mL) in control group (G1), imiclopramid group (G2), olive oil before imiclopramid group (G3) and olive oil after imiclopramid group (G4). Different symbols indicate significant differences between groups at P≤0.05 vs the control group G1 (*); vs G2 (#); between G3 and G4 ($).

DISCUSSION

In the current study, DNA damage was detected by comet assay and revealed a significant increase in TL, tail DNA%, TM, and OTM by the 8th and 12th weeks in IMI exposed group when compared with the control group. A previous study found that the percentage of the comet DNA in rats significantly increased when exposed to IMI at a dose of 1 mg/kg for 65 days (Mohamed *et al.*, 2017). IMI acts as an alkylating agent with electronegativity (Jansen *et al.*, 1994) due to the presence of an electronegative pharmacophore in the nitroguanidine moiety of the neonicotinoids such as IMI, which can bind to the DNA bases and form adducts leading to DNA strand breaks (Tomizawa *et al.*, 2003).

The obtained results revealed a significant decrease in TL, tail DNA%, TM, and OTM in OLO before IMI group compared with IMI group by the 8th and 12th weeks. There was a significant decrease in OLO after IMI group compared with IMI exposed group for TL and for OTM by the 8th and 12th weeks, for tail DNA% by the 12th week and for TM by the 8th week. Moreover, tail DNA % significantly decreased in OLO before IMI group compared with OLO after IMI group by the 8th week. This corroborates with Quiles *et al.* (2002) who revealed the lifelong OLO feeding in rats reduced DNA damage and increased antioxidant capacity and improved plasma lipid profile. Genotoxicity and apoptosis were decreased by hydroxytyrosol through downregulation of phosphoprotein p53, bax, cytochrome c, and caspase 3 and upregulation of prosurvival proteins such as the nuclear factor erythroid 2-related factor 2 and metallothionein (Mohan *et al.*, 2015). The cytoprotective effect of OLO contributes to the anti-genotoxic and anti-apoptotic properties of hydroxytyrosol (Officioso *et al.*, 2016). Also, extra virgin olive oil lessens the genotoxic and apoptotic effects of 5-5-hydroxymethylfurfural and protects the liver due to its potent antioxidant and good nutraceutical properties (El Bohi *et al.*, 2020). Moreover, the mixture of olive leaves and selenium in the diet of growing rabbits decreases DNA damage in leukocytes (Mattioli *et al.*, 2020).

The results from the present study indicated that supplementation with OLO reduced the DNA damage induced by IMI. In addition, pre-treatment with OLO was the most effective in decreasing damage. The biochemical analysis of serum MDA level revealed a significant increase in IMI exposed groups over the whole period of the experiment. This result coincides greatly with data of Duzguner & Erdogan (2010) who repor-

ted that exposure to IMI at a dose of 2.6 mg/100 g b.w. in rats resulted in a significant increase in plasma MDA concentration. Pesticides induce ROS formation (Gultekin *et al.*, 2001). The interactin of these ROS with cellular membrane result in lipid peroxidation as reported by Kapoor *et al.* (2011) and finally, cellular deterioration and oxidative stress (Khan & Kour, 2007).

In the current study, there was a significant decrease in MDA level in OLO before IMI group and OLO after IMI group than in IMI exposed group by the 8th and 12th weeks, in line with the findings of Mokhtari *et al.* (2020) affirming that virgin olive oil increased antioxidant enzymes levels and decreased MDA content of renal tissue following sub-chronic exposure to ethephon in male rats. Phenolic compounds of olive oil are located at the surface of the phospholipid bilayer in the cell membrane to chelate peroxyl radicals and to regenerate alpha-tocopherol (Paiva-Martins *et al.*, 2003). Consequently, they enhance the antioxidant defense mechanism and increase the resistance of lipid and protein to oxidation (Ghorbel *et al.*, 2015).

In the present study, GPx showed a significant decrease in IMI exposed group over the whole period of the experiment vs untreated controls. This is compatible with data from Lohiya *et al.* (2017) who reported that the activity of GPx significantly decreased in Wistar albino rats exposed orally daily to 38 mg/kg IMI for 20 and 30 days. Also, exposure to IMI at 20 mg/kg/day for 90 days resulted in a decrease of GPx activity in rats (Kapoor *et al.*, 2011). Toxic effects of IMI contribute to excess production of ROS, resulting in alterations in the cellular antioxidant defense system and affecting susceptibility to oxidative stress (Lonare *et al.*, 2016).

GPx activity in OLO before IMI group and OLO after IMI group indicated a significant increase over the whole period of the experiment when compared with IMI exposed group. Moreover, a significant increase was recorded in OLO before IMI group by the 12th week when compared with OLO after IMI group. This was in agreement with Nakbi *et al.* (2010) who reported that the treatment of rats with OLO or hydrophilic extract in association with 2, 4-dichlorophenoxyacetic acid increased the level of GPx. The antioxidant activity of biophenols contributes to the number of hydroxyl groups in the molecule. Hydroxytyrosol has two hydroxyl groups and tyrosol has only a single 4-hydroxyl group; therefore they play an important role in antioxidant function. The transcription of antioxidant responsive elements, which are found in the promoters of many genes can be stimulated by dietary OLO polyphenols (Masella *et al.*, 2005).

CONCLUSION

Imidacloprid induced DNA damage in the bone marrow and biochemical changes in serum MDA and GPx activity. Exposure to olive oil as a protective or therapeutic agent resulted in improvement in the these parameters but did not completely revert them to normal.

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