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Extra virgin olive oil polyphenols improve hematological parameters and mineral profile disorders in rats exposed to a high dose of acrylamide

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Abstract

Our study investigated the protective efficacy of extra virgin olive oil polyphenols against disorders in blood hematological parameters and mineral profile in ratsexposed to a high dose of acrylamide.

Animals were divided into four groups of six each: group 1, serving as controls, received distilled water; group 2 received ACR (50 mg/kg body weight) by gavage; group 3 received both acrylamide and olive oil polyphenols (1ml) by gavage; group 4 received only polyphenols (1 ml) by gavage for 3 weeks.

ACR-treated group showed significant differences in several hematological parameters including red and white blood cellscount, hemoglobin concentration, hematocrit value and platelet count. Moreover, there were changes in the plasma levels of some minerals such as iron, calcium and magnesium,while the phosphorus levels remained unchanged when compared to those of the control group. Supplementation of olive oil polyphenols restored changes in blood hematological and mineral profiles to near normal values. In conclusion, polyphenols might act as protective agents in preventing ACR-induced disturbances in bloodcell parameters and mineral profile.

Keywords: Acrylamide, rats, olive oil polyphenols, hematological and mineral profiles.

1. Introduction

Acrylamide (ACR) is considered as a food toxicant found in carbohydrate-rich foods with low protein content, including fried potatoes, potato chips, coffee and cereals. It can be produced naturally in carbohydrate-rich foods as a consequence of a Maillard reaction between the carbonyl group of reducing sugars and asparagine in a wide range of human foods processed at high temperature (Stadler *et al.*, 2002). Once inside the body, ACR can be conjugated with glutathione or oxidized by CYP2E1

to form the more reactive epoxide, glycidamide (Capuano *et al*., 2011). Both ACR and its metabolite glycidamide can interact with hemoglobin to yield hemoglobin adducts. Our previous studies have indicated that ACR mediates intracellular oxidative stress by promoting generation of reactive oxygen species (ROS), leading to the depletion of antioxidant status and DNA damage (Ghorbel *et al*., 2015).It is well known that oxidative stress and mitochondrial dysfunction have a main role in ACR-induced toxicity (Jiang *et al*., 2018). ACR induces toxic effects in humans and animals, such as neurotoxicity, reproductive toxicity, hepatotoxicity, immunotoxicity, genotoxicity and carcinogenicity (Kunnel *et al*., 2019). Toxicity can be prevented by synthetic or natural antioxidants.

Extra virgin olive oil (EVOO), considered as a natural antioxidant, has been recognized for its antioxidant properties and its positive effects against oxidative stress by scavenging free radicals and breaking radical chain capacity (Fito *et al*., 2007). It is the main fatty source responsible for many human health benefits due to its dietary pattern (Tsartsoum *et al*., 2019). Polyphenols, bioactive molecules in EVOO, are considered as free radical scavengers against environmental stress (Cicerale *et al*., 2018). Phenolic compounds occur in the form of phenolic acids or alcohols, oleuropein derivatives, lignans, and flavonoids. Oleuropein and hydroxytyrosol are the most important active phenols present in EVOO (Hohmann *et al*., 2015).

Interestingly, oleuropein has been reported to inhibit aromatase, a cytochrome P450 enzyme, which is an important pharmacological target in breast cancer therapy (Neves *et al*., 2007). Hydroxytyrosol is a ROS scavenger that reduces LDL oxidation and platelet aggregation (Correra *et al*., 2009). Phenolic compounds of olive oil like hydroxytyrosol, oleuropein, and verbascoside protect Caco-2 cells from oxidative stress by inhibiting ROS generation and reducing membrane oxidative damage (Serreli *et al*., 2017).

The present study evaluated the protective efficacy of extra virgin olive oil polyphenols against disorders in blood hematological and mineral parameters induced by a high dose of ACR in rats.

2. Materials and methods

2.1. Oil samples

Biologic extra virgin olive oil (EVOO) samples were obtained from a *Chetoui* variety cultivar grown in the city of Sfax, Tunisia. The hydrophilic fraction (OOHF) was extracted from EVOO by the method of Montedoro *et al*. (1992) using water instead of methanol to avoid its toxic effect in rats. Briefly, 10 g of EVOO was homogenized with 10 mL of water by a mixer (Ultra-Turrax T25 [IKA Labortechnik, Janke& Kunkel, Staufen, Germany]; 15 000×g/min) and centrifuged at 5000 × g for 10 min. The extraction was performed two times.

2.2. Total polyphenol content

As reported by Montedoro *et al*. (1992), the phenolic compounds were extracted, estimated colorimetrically at 765 nm using the Folin-Ciocalteau reagent, and expressed as hydroxytyrosol equivalents.

2.3. Animals and treatments

Twenty-four female Wistar rats, weighing 160 ± 10 g, were obtained from the Central Pharmacy (SIPHAT, Tunisia). They were housed at ambient temperature (22 ± 2°C) in a 12-h light/dark cycle and a minimum relative humidity of 40%. Food (SNA, Sfax, Tunisia) and water were available *ad libitum*. After one week of acclimatization to laboratory conditions, the rats were randomly divided into four groups of six each:

- Group 1: served as a negative control group, where rats received daily distilled water.
- Group 2: rats received daily by gavage ACR at a dose of 50 mg/Kg body weight (bw).
- Group 3: rats were exposed to ACR and co-administered daily with 1 ml of the hydrophilic fraction (OOHF) by gavage.
- Group 4: served as a positive control group, where rats received daily 1 ml of OOHF by gavage.

The dose of ACR (50 mg/ Kg bw) caused toxicity without lethality according to Rivadeneyra-Domínguez *et al*. (2018). Treatments lasted three weeks.

2.4. Blood samples preparation

All rats were euthanized by cervical decapitation to avoid stress. The trunk blood was collected. One fraction of blood was immediately collected into EDTA-containing tubes for full blood count. Another fraction, collected in heparin-coated tubes, was centrifuged at 2200 x g for 10 min to obtain plasma samples for analysis of mineral parameters.

2.5. Evaluation of hematological parameters

Total red (RBC), white (WBC) and platelet (Plt) blood cells count, hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and mean corpuscular hemoglobin (MCH)values and mean corpuscular hemoglobin concentration (MCHC) were determined with the use of an automatic hematological assay analyzer (Beckman Coulter, USA).

2.6. Evaluation of mineral parameters

Plasma levels of iron, calcium, phosphorus and magnesium were assayed spectrophotometrically using commercially available diagnostic kits (Biomaghreb, Tunisia, Ref 20064, 20051, 20084, 20074, respectively).

2.7. Statistical analysis

The data were analyzed using the statistical package program Statview 5 Software for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test as a post hoc test for comparison between groups. Student unpaired t-test was also used when comparison between two groups was required. All values were expressed as means ± SD. The 0.05 level was selected as the point of minimal statistical significance.

3. Results

3.1. Total polyphenols content

As shown in Table 1, the hydrophilic fraction of *Chetoui* olive oil contained high amounts of phenolic compounds (365.25 ± 16.22 mg/ kg).

Table 1. Total polyphenols content of the hydrophilic fraction (OOHF) of extra virgin olive oil

	Total polyphenols content(mg/kg)
OOHF	365.25 ± 16.22

3.2. Hematological parameters

As shown in Table 2, a significant decrease ($P<0.05$) in RBC and Plt numbers, Hb concentration and Ht value was observed following rats' exposure to ACR as compared to their corresponding controls. No changes in MCV, MCH, and MCHC were observed, while an increase in WBC counts by 14%, was noted in ACR-exposed rats compared to the controls. The supplementation of OOHF to ACR-treated rats ameliorated these hematological parameters.

Table 2. Hematological parameters of control and treated rats with ACR, OOHF or their combination (ACR + OOHF)

Values were expressed as means ± SD.

WBC: White Blood Cells; Plt: platelets; RBC: Red Blood Cells; Hb: hemoglobin; Ht: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular hemoglobin concentration.

ACR, (ACR + OOHF) groups vs. control group: *P<0.05; **P<0.01; ***P<0.001.

(ACR + OOHF) groups vs. ACR group: ++P<0.01; +++P<0.001.

Parameters and treatments	Controls	ACR	ACR + OOHF	OOHF
WBC count ($10^3/mm^3$)	12.45 ± 0.87	14.59 ± 0.48 ***	12.78 ± 0.43 +++	12.33 ± 0.88
Plt($10^3/\mu l$)	760± 30.18	512 ± 25.55 ***	711± 40.14 +++	740± 36.62
RBC count ($10^6/mm^3$)	7.98 ± 0.09	5.72 ± 0.39 ***	8.12 ± 0.22 +++	7.76 ± 0.11
Hb(g/dL)	14± 0.88	11± 0.58 ***	13± 0.29 *+++	14± 0.61
Ht(%)	45± 1.01	42± 0.15 **	44 ± 1.01 ++	44 ± 0.88
MCV (mm^3/RBC)	52 ± 1.17	52 ± 1.13	52 ± 0.98	53 ± 0.89
MCH (pg/RBC)	16 ± 0.51	17 ± 0.66	16 ± 0.48	17± 0.19
MCHC (g/dL)	31 ± 1.05	32 ± 0.79	32± 1.13	32± 0.74

3.3. Plasma mineral parameters

In the treated group, the plasma iron, calcium and magnesium levels were significantly decreased ($P<0.05$), while there were no changes in phosphorus levels when compared to those of control group (Table 3). Interestingly, OOHF was found to alleviate the changes of mineral profile in ACR-treated rats by enhancing plasma trace elements to near normal values.

Table 3. Iron, calcium, phosphorus and magnesium plasma levels in control and treated rats with ACR, OOHF or their combination (ACR+OOHF)

Values were expressed as means ± SD.

ACR, (ACR + OOHF) groups vs. control group: *P<0.05; **P<0.01; ***P<0.001.

(ACR + OOHF) groups vs. ACR group: ++P<0.01; +++P<0.001.

(ACR + OOHF) groups vs. ACR group: ++P<0.01; +++P<0.001.

Parameters and treatments	Controls	ACR	ACR + OOHF	OOHF
Iron (µmol/L)	33.78 ± 1.11	22.49 ± 1.02 ***	29.79 ± 1.24 **+++	32.69 ± 1.56
Calcium (mmol/L)	2.11 ± 0.12	1.81 ± 0.15 **	2.09 ± 0.13 ++	2.15 ± 0.21
Phosphorus (mmol/L)	2.68 ± 0.22	2.72 ± 0.11	2.61 ± 0.33	7.51 ± 0.18
Magnesium (mmol/L)	0.61 ± 0.10	0.54 ± 0.08 ***	0.58 ± 0.08 *++	0.60 ± 0.05

4. Discussion

Humans are primarily exposed to ACR at low concentrations chronically through the increased intake of starchy foods. To our knowledge, findings concerning the effects of this contaminant administered at a high dose to rats on blood hematological and mineral profiles are scarce. Hence, the aim of the present work was to elucidate the beneficial effects of olive oil polyphenols against ACR-induced hematological and mineral profile disorders in rats at a dose of 50 mg/kg bw.

ACR accumulates at higher levels in the blood more than any other tissue following exposure via oral ingestion, inhalation, or via the dermis (Shipp *et al*., 2006). Blood is the most important body fluid governing vital functions such as respiration, circulation, digestion, excretion, and the transport of metabolic substances. Hematological assessment provides useful information about the toxicity of contaminants (Alghamdi and El-Ghazaly, 2012). Our data showed abnormalities in some blood cell parameters of ACR-treated group. Thus, we observed a decrease in the erythrocytes and platelets count, Ht percentage and Hb level which indicated the occurrence of microcytic anemia. Red blood cell hemolysis reflects the degradation of cell integrity that can contribute to intracellular hemoglobin leakage (Eid *et al*., 2015). Our results were in agreement with those reported by Rivadeneyra-Domínguez E *et al*. (2018) in rats treated with ACR at 25 and 50 mg/kg bw. ACRisan electrophile which covalently binds to the cysteine residues and forms adducts with the sulfhydryl groups on hemoglobin resulting in the loss of its heme. Consequently, the amount of Hb in the blood is reduced which could lead to anemia (Barber *et al*., 2001).The MCV, MCH, and MCHC parameters were unchanged. Treatment of rats with ACR induced a significant increase in WBC counts, which might indicate the activation of immune system reflecting the incidence of inflammation. We have demonstrated in a previous work that administration of a low dose of ACR

(20mg/kgbw) to rats activated the inflammatory cells and amplified the expression of pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Ghorbel *et al*., 2015). ACR and its epoxide glycidamide form adducts with the NH_2 group of the valine residue of Hb, which could disturb serum iron concentrations (Konings *et al*., 2003). In the present study, we observed a marked fall in plasma iron level after ACR administration. Other minerals like calcium and magnesium were slightly reduced following ACR treatment. The phosphorus level was unchanged. It has been reported that exposure to ACR decreased serum zinc, selenium, cobalt and magnesium concentrations (Yerlikaya *et al*., 2013). ACR-induced oxidative stress and deteriorating effects on enzyme activities influence the ability of membranes to control ion movements between cellular compartments.

The present study revealed that the administration of olive oil containing a high amount of polyphenols to ACR-treated rats induced a significant recovery of RBC and Plt numbers, Hb concentration and Ht value which could be attributed to the free radical scavenging activity of polyphenols. Additionally, co-administration of OOHF decreased significantly WBC counts. Indeed, EVOO phenolic compounds have been widely recognized as anti-inflammatory agents for their capacity to modulate improper activation of the NF-kB signaling pathway, thus limiting its deleterious effects in all tissues. According to Bajoub *et al*. (2017), the most abundant phenolic compounds present in olive oil can be classified as follows: secoiridoids (oleuropein, oleuropeinaglycone, oleacanthol), simple phenols (tyrosol, hydroxytyrosol), lignans (pinoresinol, syringaresinol), flavonoids (luteolin, apigenin) and phenolic acids. The consumption of olive oil highly concentrated in phenols (398 ppm of phenols like hydroxytrysol, tyrosol, and minor components) significantly limits the activation of NF-kB postprandial gene expression in peripheral blood mononuclear cells in humans (Camargo *et al*., 2014). In fact, alterations in hematological parameters are considered as the crucial biomarkers of physiological stress (Akinrotimi *et al*., 2009). Due to their potent antioxidant power, natural polyphenols decrease the level of ROS, contributing consequently to the protection of biomolecules against oxidative damage. An ameliorative action of olive oil on the plasma levels of iron, calcium and magnesium in ACR-treated rats was also noted in the present work. In line with our findings, Cardoso *et al*. (2020) have shown an increase in the serum level of calcium in severely obese adults who have received extra virgin olive oil in their diet.

5. Conclusion

Our study demonstrated that EVOO polyphenols showed beneficial effects against ACR-induced changes in blood hematological and mineral profiles in rats. These effects might be related to their radical scavenging properties and their effect as a regulator of antioxidative systems. Olive oil polyphenols might provide a basis for developing a new dietary supplementation strategy and therapeutic approach in order to prevent abnormalities in blood cell parameters and mineral profile disturbances.

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Conflict of Interest Statement
The authors declare that they have no competing interests to disclose.

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