Ameliorative effect of extra virgin olive oil on hexavalent chromium-induced nephrotoxicity and genotoxicity in rats

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SUMMARY

This study investigated the ameliorating effect of extra virgin olive oil (EVOO) on hexavalent chromium [Cr (VI)]-induced nephrotoxicity and genotoxicity in rats. Twenty-four male Sprague-Dawley rats were divided into four groups of six animals (n= 6/group): group I served as a control, group II received oral Cr (VI) at a dose of 6.25 mg/Kg body weight (BW), group III was co-treated with 6.25 mg/Kg BW Cr (VI) and 1.5 ml/Kg BW EVOO, and group IV was orally administered EVOO (1.5 ml/Kg BW) three times a week for 4 weeks. The results showed that Cr (VI) significantly (p<0.05) increased the levels of serum urea, creatinine, total protein, and albumin; the albumin/globulin (A/G) ratio; and the renal malondialdehyde (MDA) concentration. In addition, Cr (VI) significantly decreased catalase (CAT) and superoxide dismutase (SOD) activities and reduced glutathione (GSH) levels when compared with the control group. Cr (VI)-treated rats showed a significant increase in all parameters of kidney DNA damage using a comet assay. Co-treatment with EVOO ameliorated all of the Cr (VI)-induced responses listed above. These results were corroborated with histopathological findings. The present study suggests that EVOO is effective at ameliorating some of the kidney and DNA damage induced by Cr (VI) toxicity. Further studies are needed to evaluate this ameliorative effect of EVOO on other environmental pollutants.

Keywords: Hexavalent chromium, Antioxidant, Nephrotoxicity, Genotoxicity, Extra virgin olive oil.

RESUME

Effet de l'huile d'olive extra vierge sur la néphrotoxicité et la génotoxicité induite par le chrome hexavalent chez le rat

Cette étude a examiné l'effet de l'huile d'olive extra vierge (EVOO) sur la néphrotoxicité et la génotoxicité induites par le chrome hexavalent [Cr (VI)] chez le rat. Vingt-quatre rats Sprague-Dawley ont été divisés en quatre groupes de six animaux (n = 6 / groupe): le groupe I a servi de témoin, le groupe II a reçu le Cr (VI) par voie orale à une dose de 6,25 mg/kg de poids corporel (pc), le groupe III était co-traité avec EVOO à la dose de 1,5 ml/kg pc et Cr (VI) à la dose de 6,25 mg/kg pc, et le groupe IV a été traité avec EVOO à la dose de 1,5 ml/kg pc. Les traitements ont été administrés trois fois par semaine pendant quatre semaines. Les résultats ont montré que l'administration de Cr (VI) a augmenté de manière significative (p <0,05) les niveaux sériques de l'urée, de la créatinine, des protéines totales et de l'albumine ainsi que le rapport albumine/globulin (A/G); et la concentration rénale en malondialdéhyde. De plus, l'administration de Cr (VI) a diminué de manière significative les activités de la catalase et de la superoxyde dismutase et les teneurs en glutathion réduit par rapport aux témoins alors qu'une augmentation significative de tous les paramètres utilisés pour mesurer la présence de dommages à l'ADN, en utilisant un test des comètes, étaient augmentées. L'administration conjointe de l'EVOO a diminué tous les effets induits par le Cr (VI). Ces résultats ont été corroborés par analyses histopathologiques.

Mots-clés: chrome hexavalent, antioxydant, néphrotoxicité, génotoxicité, huile d'olive extra vierge.

Introduction

Chromium (Cr) is one of the most toxic chemical compounds because of its increased level in the environment as a result of industrial and agricultural practices. Chromium is a significant environmental pollutant that is abundant in aquatic and terrestrial ecosystems [10]. Chromium exists as hexavalent chromium [Cr (VI)] and trivalent chromium [Cr (III)]. Chromium (VI) is commonly used in several industrial processes, including chrome plating, welding, painting, textile manufacture, metal finishing, manufacture of stainless steel and wood preservation [4]. Intracellular reduction of Cr (VI) to Cr (III) causes an overproduction of reactive oxygen species (ROS), which is an important characteristic of Cr (VI) toxicity [44].

Human exposure to Cr (VI) induces several adverse health effects, such as genotoxicity, nephrotoxicity, carcinogenicity and hepatotoxicity [14, 37, 43]. Potassium dichromate (K2Cr2O7) is the most toxic form of Cr (VI), and it has been found to induce nephrotoxicity [1, 34] and DNA damage [22] in experimental animals.

Extra-virgin olive oil (EVOO) is an important component of daily diets because of its significant health benefits. These benefits include reducing DNA oxidation and positively influencing cholesterol regulation and oxidation of low-density lipoprotein. In addition, EVOO has anti-inflammatory, antithrombotic, antimicrobial, anticarcinogenic, antihypertensive, and vasodilatory effects [26].

The antioxidant properties of olive oil-derived phenolic compounds (oleuropein and hydroxytyrosol) are linked with inhibition of lipid peroxidation and free radical scavenging activity [45]. However, to our knowledge, there is a lack of literature available on the ameliorative effect of EVOO on Cr (VI)-induced nephrotoxicity and genotoxicity. Therefore, the objective of this study was to evaluate the efficacy of...
EVOO at ameliorating Cr (VI)-induced toxicity in rats based on renal function markers in the serum, antioxidant enzyme activities, histopathological changes in the kidney (light microscopy) and DNA damage detected by a comet assay.

**Materials and Methods**

**CHEMICALS AND REAGENTS**

Potassium dichromate (K\(_2\)Cr\(_2\)O\(_7\)) was obtained from the El-Gomhoria Chemical Company in Egypt as a yellow powder dissolved in distilled water. EVOO was produced by Angle Camacho Alimentacion, S.L., Spain and purchased from a local market. Other chemicals were obtained from Sigma (St. Louis, MO, USA).

**ANIMALS AND EXPERIMENTAL PROTOCOL**

Twenty-four healthy adult male Sprague-Dawley rats weighing 200±10 g were obtained from the Animal Research Unit within the Faculty of Veterinary Medicine at Zagazig University in Egypt. The animals were acclimated to laboratory conditions for 2 weeks before the start of the experiment. Rats were kept in metal cages during the experimental period and maintained on a 12-h light-dark cycle with controlled temperature (21–24 °C) and relative humidity (50–60%). Animals were given a standard diet and water ad libitum. All animals were treated in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals, and the protocols were approved by the ethics of animal use in research committee (EAURC), Cairo University. The animals were randomly divided into four equal groups (six per group). Group I (control group) received no treatment. Group II (Cr (VI)-treated group) was orally administered K\(_2\)Cr\(_2\)O\(_7\) dissolved in distilled water at a dose of 1/8 LD\(_{50}\) (6.25 mg/kg BW) 3 times a week for 4 weeks. Group III (Cr (VI) + EVOO-treated group) rats were co-treated with K\(_2\)Cr\(_2\)O\(_7\) (6.25 mg/kg BW) and EVOO (1.5 ml/Kg BW) [28] orally three times a week for 4 weeks. Group IV (EVOO administered group) was gavaged with EVOO (1.5 ml/Kg BW) 3 times a week for 4 weeks.

At the end of the experiment, blood samples were collected from the rat median canthus of all control and treated groups. The samples were left to clot over night at room temperature, then centrifuged at 3000 rpm for 15 min. The resulting supernatant was used to estimate the oxidative stress markers. The CAT activity was measured from the rate of dismutation of hydrogen peroxide to water and molecular oxygen in a two-step coupling reaction [2]. The SOD activity was estimated according to NISHIKIMI et al. [29]. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye. While, GSH level was assayed from the reduction of 5,5’-dithiobis (2-nitrobenzoic acid). The chromogen formed is directly proportional to the GSH concentration [8]. The MDA concentration was determined according to OHKAWA et al. [32]. This was based on the formation of thiobarbituric acid reactive substances (TBARs).

**EVALUATION OF DNA DAMAGE AND HISTOPATHOLOGICAL EXAMINATION**

The DNA damage in kidney was estimated using single cell gel electrophoresis (SCGE); comet assay. Briefly, a portion of the kidney specimen from treated and control groups was minced with an ice-cold mincing solution (Ca\(^2+\) and Mg\(^2+\)-free HBSS containing 20 mM EDTA and 10% DMSO) in a Petri dish. The cell viability was estimated by analyzing comet images after electrophoresis [12]. Samples were cut into smaller pieces using a disposable microtome razor blade. Next, the solution was aspirated and a fresh mincing solution was added. Samples were minced again into smaller pieces. The resulting cell suspensions were collected and filtered (100-μm nylon mesh). Samples were stored covered on ice to avoid light until the comet assay procedure commenced.

The comet assay was carried out under alkaline conditions according to a previously described standard protocol [9]. Comet images were analyzed using a visual scoring method and computerized image analysis [24]. To quantify DNA damage, tail length, tail DNA (%) and tail moment were analyzed using Comet Assay Project Software (CAPS). For the majority of the samples, 50-100 randomly selected cells were analyzed.
For histopathological examination, kidney specimens were fixed in 10% neutral-buffered formalin (pH 7.4), dehydrated and embedded in paraffin. Sections (4-µm thickness) were stained using hematoxylin and eosin (HE) [6].

**STATISTICAL ANALYSIS**

The data were expressed as the mean ± standard error (SE). The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the post-hoc Duncan’s test for comparison between different experimental groups. We used IBM SPSS Statistics computer software (version 21). P-values < 0.05 were considered statistically significant.

**Results**

**EFFECT OF CR (VI) AND CO-TREATMENT WITH EVOO ON RENAL FUNCTION AND OXIDATIVE STRESS MARKERS**

Rats treated with Cr (VI) showed a significant increase in serum urea and creatinine levels when compared with the control group. The group co-treated with Cr (VI) and EVOO showed a significant decrease in these enzymes when compared with Cr (VI)-treated rats (Table I).

The Cr (VI)-treated group showed a significant decrease in total protein, albumin and A/G ratio when compared with the control group. Co-administration of EVOO with Cr (VI) increased serum total protein, albumin and the A/G ratio when compared with the Cr (VI)-treated group (Table I).

When compared with the control group, the renal MDA concentration was significantly increased and the GSH concentration and CAT and SOD activities were significantly decreased in rats treated with Cr (VI) when compared with control group. In contrast, rats co-treated with Cr (VI) and EVOO showed a significant decrease in MDA concentration and an increase in GSH content and CAT and SOD activities when compared with the Cr (VI)-treated rats (Table II).

**EFFECT OF CR (VI) AND CO-TREATMENT WITH EVOO ON OXIDATIVE DNA DAMAGE IN THE KIDNEY**

The results showed that the Cr (VI)-treated group had a significant increase in all parameters of DNA damage in the kidney (tail length, DNA tail % and tail moment) when compared with the control group. Co-treatment with EVOO ameliorated the DNA damage to the kidney by significantly decreasing tail length, DNA tail % and tail moment when compared with the Cr (VI)-treated group (Figure 1 and Table III).

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EFFECT OF CR (VI) AND CO-TREATMENT WITH EVOO ON THE HISTOPATHOLOGY OF THE KIDNEY

Light microscopy of HE stained kidney sections from control and EVOO-administered groups revealed normal histological structures (Figure 2, A). The kidney of Cr (VI)-treated rats showed severe congestion and hemorrhages within the renal tubules. Multifocal areas of coagulative necrosis and vacuolations were detected in the epithelial lining of the renal tubules as shown by pyknosis and karyolysis. Congested glomerular capillaries, hyaline droplet degeneration and necrosis in the renal tubular epithelium were also visualized (Figure 2; B1). Additional glomeruli showed shrunken glomerular tufts, necrosis and vacuolation in the renal epithelia and congested peritubular capillaries (Figure 2; B2). Focal interstitial, perivascular and periglomerular aggregation of mononuclear cells were predominately observed for lymphocytes (Figure 2; B3). Atrophy and atresia of a portion of the medullary renal tubules with numerous lymphocyte infiltrations (Figure 2; B4) were observed; in addition, dilated renal tubules were detected. The lesions in the Cr (VI) +EVOO group were markedly reduced when compared with the Cr (VI)-treated group. The kidneys of Cr (VI) +EVOO treated rats showed slight cloudy swelling and vacuolation in the proximal convoluted tubules and hypercellularity of the glomerular tufts. The congestion and perivascular edema infiltrated with lymphocytes was reduced and a few round cells were observed among the renal tubules (Figure 2; C).

Discussion

There was no mortality in this study, which agrees with the LD$_{50}$ of chromium reported to be 50 mg/kg BW in rats [4]. For estimation of A/G ratio in this study, the serum globulin was calculated by subtracting the serum albumin value from the value of serum total protein. Our results showed that rats administered Cr (VI) at a dose of 6.25 mg/kg BW elicited a significant (p<0.05) increase in serum levels of urea and creatinine with a significant decrease in serum total protein, albumin and A/G ratio when compared with the control group. The significant reduction in serum total...
protein may be attributed to proteinuria, nephropathy and an increase in amino acids deamination [3, 40]. The impaired kidney functions observed in this study are consistent with previous studies [5, 27, 48].

The current results may be attributed to the fact that Cr (VI) compounds are easily taken up by the cells and reduced to Cr (III). In turn, this reduction generates free radicals that play a major role in the adverse biological effects of Cr (VI) [41]. The oxygen free radicals are considered to be important mediators of Cr (VI)-induced renal damage because the kidney is the major target organ of toxicity and is more vulnerable to damage due to perfusion and the increased concentration of excreted compounds in renal tubular cells [7]. The remaining parameters in the present work corroborate these suggestions because we observed alterations in the activities of antioxidant enzymes along with lipid peroxidation, DNA damage and histopathological changes in kidney of K$_2$Cr$_2$O$_7$-administered rats.

Our results revealed that K$_2$Cr$_2$O$_7$ treatment in rats induced renal oxidative damage by significantly (p<0.05) elevating MDA levels, which is a marker of lipid peroxidation. We also found a concomitant decrease in antioxidant enzyme activity (CAT and SOD) and GSH concentration. These findings are concordant with previous reports [15, 23, 34].

Cr (VI)-induced oxidative stress may be attributed to its ability to generate reactive oxygen species (ROS) through cellular reduction of Cr (VI) into Cr (III) leading to oxidative damage of renal tissues and lipid peroxidation [11]. ROS includes the superoxide anion radical (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$) [25]. An increase in H$_2$O$_2$ can induce peroxidation of polyunsaturated fatty acids resulting in impaired membrane function, structural integrity, decreased membrane fluidity and inactivation of several membrane bound enzymes [20]. This is confirmed by the significant increase in MDA levels and depletion of antioxidant enzyme activities in the present study. These parameters are considered to be the body’s primary defense against oxidative injury to biological macromolecules. These enzymes also remove peroxides, free radicals and superoxide anions generated inside the cell due to their free radical scavenging properties [42]. The majority of antioxidant
enzymes become inactive after exposure to $\text{K}_2\text{Cr}_2\text{O}_7$, either due to direct binding of heavy metals to the active site of the SH group-containing enzyme or due to the displacement of metal co-factors from active sites [36]. The antioxidant defense system of the body antagonizes the onset of ROS via GSH, which is the most abundant non-protein thiol endogenous antioxidant. A reduction in GSH levels may impair the clearance of $\text{H}_2\text{O}_2$, and enhance the formation of OH, the most toxic radical, leading to oxidative damage. CAT is also involved in the detoxification of $\text{H}_2\text{O}_2$, by its decomposition into water and oxygen [39]. SOD catalyzes the dismutation of $\text{O}_2^-$ and formation of $\text{H}_2\text{O}_2$ and protects cells from superoxide toxicity [38]. This study found a significant reduction in the antioxidant enzyme reserve and the elevated lipid peroxidation in the kidney that suggests a failure of antioxidant defense system to overcome the influx of ROS generated by $\text{K}_2\text{Cr}_2\text{O}_7$.

Previous studies have shown that rats orally administered with natural antioxidants, such as garlic powder and curcumin, were protected against $\text{K}_2\text{Cr}_2\text{O}_7$-induced nephrotoxicity associated with oxidative stress [27, 37]. However, few studies are available on EVOO as a natural antioxidant for ameliorating Cr toxicity.

Notably, the oral administration of EVOO to rats treated with Cr (VI) ameliorated the Cr (IV)-induced renal dysfunction, as indicated by a significant restoration of renal function biomarkers. Concomitant treatment of EVOO with $\text{K}_2\text{Cr}_2\text{O}_7$ significantly ameliorated the reduction in GSH concentration and CAT and SOD activities, but it did not restore values to the control levels. Additionally, the level of lipid peroxidation was significantly decreased in rats co-treated with EVOO and Cr (VI) when compared with those treated with Cr (VI). This study confirmed that EVOO improves Cr (VI)-induced oxidative stress by increasing the activity of antioxidant enzymes and reducing MDA and GSH concentrations. This effect may be attributed to the presence of tyrosol and hydroxytyrosol which is abundantly present in olive oil as a natural polyphenol that protects biological membrane integrity. This phenolic fraction has antioxidant properties and can scavenge peroxyl radicals and other reactive nitrogen species, break peroxidative chain reactions and prevent the production of ROS catalyzed by metal ions [26]. In addition to tyrosol and hydroxytyrosol, EVOO contains a considerable amount of oleuropein and caffeic acid, two compounds that inhibit ROS production [16]. Similarly, oral supplementation of olive oil to ethanol-treated rats restored liver damage due to lipid peroxidation inhibition and improved antioxidant enzyme activity [21].

We found a significant increase (p<0.05) in DNA damage parameters in the kidney (tail length, DNA tail % and tail moment) in Cr (VI)-treated rats using a comet assay. Our results are in accordance with previous studies demonstrating that an intraperitoneal injection of Cr (VI) induced DNA damage in mice [17, 18]. In similar studies, the oral administration of $\text{K}_2\text{Cr}_2\text{O}_7$ in mice induced genotoxicity [11, 47]. The present data also coincide with a previous study showing that an intraperitoneal injection of $\text{K}_2\text{Cr}_2\text{O}_7$ induced DNA damage in the peripheral blood lymphocytes of rats [35].

The mechanism of Cr (VI)-induced genotoxicity may result from the intracellular reduction of Cr (VI) into Cr (III). Chromium metabolite radicals produced during this reduction could subsequently attack macromolecules and produce different forms of DNA damage, including strand breakage, DNA-protein crosslinking, DNA-DNA crosslinking, Cr-DNA adducts and base modifications in cells [31].

EVOO administration produced a significant amelioration (p<0.05) of the DNA damage caused by $\text{K}_2\text{Cr}_2\text{O}_7$. Similarly, oral administration of EVOO ameliorated Cr (VI)-induced genotoxicity in rats [22]. Moreover, these data are supported by previous findings that revealed a reduction of $\text{H}_2\text{O}_2$-induced DNA damage in different pre-treated cells with olive oil phenol extracts [19, 30].

The efficacy of olive oil to decrease the DNA damage may be due to its phenolic content that acts as a metal ion chelator and enhances the endogenous antioxidant defense system either by induction of phase II enzymes or by stimulating mitochondrial biogenesis. These effects may also be due to the DNA repair system and protection of DNA repair genes [13].

Olive oil has a high level of monounsaturated fatty acids, oleic acid and polyphenolic constituents that differ according to variety, soil, climate, oil production and storage [46]. Of interest, the EVOO in this study had a potent efficacy for the amelioration of the renal dysfunction, decreased antioxidant capacity and DNA damage in rats co-treated with Cr (VI) and EVOO. These findings may be attributed to the higher total phenol content in EVOO when compared with refined olive oil [33].

Our biochemical data are in close agreement with the histopathological findings of the kidney. The examination of the kidneys of Cr (VI)-treated rats showed extensive damage and structural abnormalities in the renal tissue, similar to the previous studies [23, 42]. The pathological lesions of the kidney induced by $\text{K}_2\text{Cr}_2\text{O}_7$ were remarkably reduced by co-administration of EVOO, suggesting that EVOO confers histopathological protection. These data support our aforementioned findings showing that concurrent administration of EVOO with $\text{K}_2\text{Cr}_2\text{O}_7$ caused a significant improvement in Cr (VI)-induced oxidative stress by increasing antioxidant enzyme activity and reducing lipid peroxidation.

In conclusion, Oral administration of Cr (VI) at a dose of 6.25 mg/Kg BW three times a week for 4 weeks resulted in impairments to renal function, DNA damage and histopathological changes in the kidney of rats. Concurrent administration of EVOO with $\text{K}_2\text{Cr}_2\text{O}_7$ ameliorated all of
these toxic effects. The ameliorative effect of EVOO may be attributed to its antioxidant and free radical scavenging properties. Our results suggest that EVOO consumption as a part of our diet may be useful in ameliorating Cr (VI)-induced nephrotoxicity and genotoxicity. Further studies are recommended to assess the ameliorative effect EVOO on other environmental contaminants.

References


