Effect of Virgin Olive Oil (Olea europea. L) on Kidney Function Impairment and Oxidative Stress Induced by Mercuric Chloride in Rats

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ABSTRACT

The study was designed to investigate the possible protective role of virgin olive oil in mercuric chloride induced renal stress, by using biochemical approaches. The effects of virgin olive oil on mercuric chloride induced oxidative and renal stress were evaluated by serum creatinine, urea and uric acid levels, kidney tissue lipid peroxidation, GSH levels, GSH-Px and GST activities. Administration of mercuric chloride induced significant increase in serum: interleukine1, interleukine6 and Tumor Necrosis Factor α (TNFα) levels, creatinine, urea and uric acid concentration showing renal stress. Mercuric chloride also induced oxidative stress, as indicate by decreased kidney tissue of GSH level, GSH-Px and GST activities along with increase the level of lipid peroxidation. Furthermore, treatment with mercuric chloride caused a marked elevation of kidney weight and decreased body weight. Virgin olive oil treatment markedly reduced elevated serum: IL1, IL6, TNFα, creatinine, urea and uric acid levels and contrated the deterious effects of mercuric chloride on oxidative stress markers changes caused by HgCl₂ in kidney. Our results indicate that virgin olive oil could have a beneficial role against mercuric chloride induced oxidative and renal stress in rat.

Keywords: Antioxidant Enzymes, Mercury, Pro-Inflammatory Cytokine, Renal Stress, Virgin Olive Oil

1. INTRODUCTION

Mercury is a well-known human and animalinduces extensive kidney damage nephrotoxicant. Acute oral or parenteral exposure induces extensive kidney damage (Fowler and Woods, 1977; Goyer et al., 1975). Studies in vivo and in vitro have demonstrated that mercury induced lipid peroxidation, suggesting the involvement of oxidative stress in its cytotoxicity (Lund et al., 1991; Stacey and Kappus, 1982). Lund et al. (1991) reported that mercury enhances renal mitochondrial hydrogen peroxide formation in vivo and in vitro. However, cansative correlation between mercury induced lipid peroxidation and cellular toxicity remains controversial. Some authors reported that lipid peroxidation plays a critical role in cell injury induced by mercury (Lund et al., 1991) in renal cells, whereas other investigators showed that lipid peroxidation is not directly responsible for mercury induced cell injury in hepatocytes and renal cells (Paller, 1985; Strubell, 1996). It is important to develop an effective drug for mercury to prevent the mercury induced cellular damages.

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Historically, plants have been used as folk medicine against various type of disease. Olive oil is an integral ingredient in the Mediterranean diet. There is growing evidence that it may have great health benefits including the reduction in coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses (Visioli and Galli, 2002; Keys, 1995; Stark and Mader, 2002). Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds (Lavelli, 2002; Visioli and Galli, 1998). It is known that an increased consumption of Monounsaturated Fatty Acids (MUFA) instead of Polyunsaturated Fatty Acids (PUFA) reduces the risk of atherosclerosis because it decreases the circulating lipoprotein’s sensitivity to peroxidation (Moreno and Mitjavila, 2003).

Furthermore, the dietary MUFA healthy effects were attributed to decreased endothelial activation (Massaro and Caterina, 2002) and LDL susceptibility to oxidation (Bononome et al., 1992). In recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and as bioactive molecules. They are particularly important inhibitors of lipid peroxidation (Salah et al., 1995) and are believed to be effective through their free radical scavenging and metal-chelating properties (Kandaswami and Middleton, 1994; Rice-Evans et al., 1996). In experimental studies, olive oil phenolic compounds showed strong antioxidant properties against lipids, DNA and LDL oxidation (Covas et al., 2006). Hydroxytyrosol (2-(3,4 dihydroxyphenyl) Ethanol, DPE), one of the phenolic compounds present in extra virgin olive oil has been suggested to be a potent antioxidant, thus contributing to the beneficial properties of olive oil (Deiana et al., 1999). DPE administration has been shown to reduce the consequences of passive smoking-induced oxidative stress (Visioli et al., 2000), prevent LDL oxidation (Wiseman et al., 1996) and platelet aggregation and inhibit leukocyte 5-lipoxygenases (De la Puerta et al., 1999). DPE has shown efficacy in preventing oxidative stress in the liver of rats intoxicated by cadmium (Casalino et al., 2002). In addition, when human hepatoma HepG2 cells were pre-treated with DPE for 2 or 20 h prior to submission to tert-butyldihydroperoxide-induced oxidative stress, cell toxicity was completely prevented, indicating that the antioxidant-treated cells were totally protected against the oxidative insult (Goya et al., 2007). However, the liver is not only the main target for phenolic antioxidants once absorbed from the gastrointestinal tract but is the major place for phenolic metabolism. Therefore, studies dealing with the effect of antioxidant dietary phenolics on the liver should be given priority. The literature data on olive oil polyphenols is mainly concerned with purified compounds, while the antioxidant properties of the total fraction of the lipophilic or hydrophilic components have been poorly investigated. Being a complex mixture of compounds, the study of the protective effect could be more representative than of a single component.

The purpose of this study was to evaluate the protective role of virgin olive oil on mercury chloride induced oxidative and renal stress in rats.

2. MATERIALS AND METHODS

The virgin olive oil used in this study originated from chetaibi (Algeria), it was extracted by a traditional method. All chemicals used in this study were purchased from sigma chemical company. Laboratory animals, Albino Wistar male rats, were brought from the Algiers Pasteur institute at the age of 8 weeks, with an average live weight of 200 g. They were located in a room with an ambient temperature of 21±1°C and up to 12 h of light daily. The rats were divided into four experimental groups; each consists of eight rats. The first group was served as the control. The second group was given virgin olive oil at a dose of 2 mL kg⁻¹ (Bouchefra and Idoui, 2012), body weight, while the third group (HgCl₂) was intraperitoneally given mercuric chloride at a dose of 0.5 mg kg⁻¹ body weight (Bensefa-colas et al., 2011). Finally, the fourth group was given combined treatment with virgin olive oil and mercuric chloride. The treatment of all groups was lasted for 2 consecutive weeks.

Twenty four hours after the last administration the blood was collected by retro- orbital sinus puncture from each anesthetized rats. After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at -20°C until determination of: IL1, IL6, TNFα, urea, creatinine and uric acid. Subsequently, rats were decapitated and kidneys were removed.

2.1. Tissue Preparation

About 500 mg of kidney was homogenized in 4 mL of buffer solution of phosphate buffered saline (w/v: 500 mg tissue with 4ml PBS, PH 7.4) homogenates were centrifuged at 10.000 xg for 15 min at 4°C and the resultant
supernatant was used for determination of: Reduced Glutathione (GSH), Thiobarbituric Acid-Reactive Substance (TBARS) level and Glutathione Peroxidase (GSH-PX) and Glutathione-S-Transferase (GST) activities.

2.2. Determination of Biochemical Parameters

Serum urea, creatinine and uric acid were determined using commercial kits (Spinreact) and serum IL1, IL6, TNFα levels were assayed using specific Elisa kits for each cytokine (Boster, immunoleader).

2.3. Determination of Lipid Peroxidation (LPO)

Lipid peroxidation level in the liver was measured by the method of Buege and Aust (1984). About 125 µL of supernatant were homogenized by sonication with 50 µL of PBS, 125 µL of 20% TCA + BHT 1% (TCA-BHT) in order to precipitate proteins and centrifuged (1000 xg, 10 min, 4°C), afterwards, 200 µL of supernatant were mixed with 40 µL of HCl (0.6 M) and 160 µL of TBA dissolved in tris (120 mM) and then the mixture was heated at 80°C for 10 min, the absorbance of the resultant supernatant was obtained at 530 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56×10^5 M/Cm.

2.4. Determination of Reduced Glutathione (GSH)

GSH content in liver was measured spectrophotometrically by using Ellman’s reagent (DTNB) as a colouring reagent, following the method described by Weckberecker and Cory (1988).

2.5. Determination of Glutathione-S-Transferase (GST) (EC2.5.1.18)

The cytosolic glutathione-S-transferase activity was determined spectrophotometrically at 37°C by method of Habig and Jakoby (1974). The reaction mixture (1 mL) contained 0.334 mL of 100 mM phosphate buffer (PH 6.5), 0.033 mL of 30 mM CDNB and 0.33 mL of reduced Glutathione. After pre-incubating the reaction mixture for 2 min the reaction was started by adding 0.01 mL of diluted cytosol and the absorbance was followed for 3 min at 340 nm. The specific activity of GST is expressed as µmole of GSH-CDNB conjugate formed/min/mg protein using extinction coefficient of 9.6 Mm^-1 cm^-1

2.6. Determination of GSH-Px (E.C.1.11.1.9)

Glutathione peroxidase (EC 1.11.1.9) activity was modified from the method of Flohe and Gunzler (1984), for the enzyme reaction, 0.2 mL of the supernatant was placed into a tube and mixed with 0.4 mL GSH (reduced glutathione, sigma product, analytical grade) and the mixture was put into an ice bath for 30 min. then the mixture was centrifuged for 10 min at 3000 rpm, 0.48ml of the supernatant was placed into a cuvette and 2.2 mL of 0.32 M Na2HPO4 and 0.32 mL of 1 m mol/L 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, sigma) were added for color development. The absorbance at wavelength 412 nm was measured with a UV spectrophotometer after 5min. The enzyme activity was calculated as a decrease in GSH within the reaction time as compared to that in the non-enzyme reaction.

2.7. Protein Quantification

Protein was measured by the method of Bradford (1976) using bovine serum albumin as the standard.

2.8. Statistical Analysis

The data were subjected to student t test for comparison between groups. The values are expressed as mean ± SEM. Significance level was set at p<0.05, p<0.01, p<0.001.

3. RESULTS

3.1. Effects of Treatments on Body, Absolute and Relative Kidney Weights

Table 1 shows the effect of mercuric chloride, virgin olive oil and combined treatment with virgin olive oil and mercuric chloride. The marked decreased in rats body weight was observed in mercuric chloride treated rats and virgin olive oil + mercuric chloride group, but the result was not significant as compared to control. Along virgin olive oil showed increased body weight but result was not significant. The kidneys of rats treated with mercuric chloride were enlarged. Mercuric chloride treated rats showed increase in kidney weight and relative kidney weight as compared to control. Combined treatment with virgin olive oil showed significant increased relative kidney weight, while alone virgin olive oil treatment had showed no significant effect.

3.2. Effects of Treatment on Serum Biochemical Parameters

A significant elevation in serum IL1, IL6, TNFα, urea, creatinine and uric acid levels was observed in mercuric chloride intoxicated rats. Only virgin olive oil treatment did not show any significant alteration. However, the combined treatment of virgin olive oil with mercuric chloride show a significant decline in serum IL1, IL6, TNFα, urea, creatinine and uric acid levels was noticed respect to controls (Table 2).
Table 1. Changes in body and absolute and relative kidney weights of control and rats treated with olive oil, mercuric chloride and combined treatment of mercuric chloride with olive oil after 2 weeks of treatment.

| Parameters                        | Control | Olive oil | HgCl₂ olive | Oil+HgCl₂ |
|-----------------------------------|---------|-----------|-------------|-----------|
| Initial body weight (g)           | 172±46.3| 176.6±45.4| 171.3±52.6  | 177.4±50.3|
| Final body weight (g)             | 175.4±75.9| 179.1±32.3| 176.1±42.2  | 173.6±41.1|
| Absolute kidney weight (g)        | 0.78±0.09| 1.02±0.12 | 0.86±0.14   | 0.88±0.20 |
| Relative kidney weight (g/100g b.w)| 0.45±0.01| 0.57±0.03 | 0.5±0.06    | 0.49±0.04 |

Table 2. Changes in biochemical parameters of control and rats treated with olive oil, mercuric chloride and combined treatment of mercuric chloride with olive oil after 2 weeks of treatment.

| Parameters                        | Control | Olive oil | HgCl₂ olive | Oil+HgCl₂ |
|-----------------------------------|---------|-----------|-------------|-----------|
| Urea (g/l)                        | 0.38±0.04| 0.37±0.02 | 0.46±0.04** | 0.45±0.03*|
| Creatinine (mg/l)                 | 2.17±0.69| 2.8±0.61  | 3.10±0.72*  | 2.91±0.60 |
| Uric acid (mg/l)                  | 25.92±10.2| 22.26±2.8 | 27.97±3.96* | 26.6±6.93 |
| IL1 (pg/ml)                       | 0.087±0.01| 0.085±0.01| 0.108±0.03* | 0.103±0.03|
| IL6 (pg/ml)                       | 0.152±0.09| 0.162±0.06| 0.217±0.18  | 0.174±0.09|
| TNF (pg/ml)                       | 0.078±0.01| 0.084±0.01| 0.293±0.26  | 0.091±0.08|

Values are given as mean ± SEM for group of 6 animals each. *P≤0.05, compared to controls. **P≤0.01, compared to controls. ***P≤0.001, compared to controls.

Fig. 1. Reduced glutathione (nmol/mg protein) and TBARS (nmol MDA/mg protein) levels in kidney of control and rats treated with olive oil, mercuric chloride and combined treatment of mercuric chloride with olive oil after 2 weeks of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: * compared to controls (*p≤0.05; **p≤0.01; ***p≤0.001)
Fig. 2. Enzyme activities of GPx (µmol GSH/mg protein) and GST (nmol/min/mg protein) in kidney of control and rats treated with olive oil, mercuric chloride and combined treatment of mercuric chloride with olive oil after 2 weeks of treatment. Values are given as mean ± SEM for group of 6 animals each significant difference: * compared to controls (*p≤0.05; **p≤0.01; ***p≤0.001)

3.3. Effects of Treatments on Renal Oxidative Stress Parameters

Mercuric chloride exposure a significant depleted in reduced glutathione level, GSH-Px and GST activities and a highly significant increase in kidney lipid peroxidation level in mercury intoxicated rats was noticed. Virgin olive oil alone treatment did not show any significant decline. Combined treatment of mercuric chloride with virgin olive oil, increase reduced glutathione level, GSH-Px and GST activities and a significant depletion in lipid peroxidation level was recorded with respect to the control (Fig. 1 and 2).

4. DISCUSSION

In the present study, oxidative stress induced by HgCl$_2$ was evidenced in kidney of rats by increase in lipid peroxidation level and the stimulation of GSH-Px, GST and catalase activities. Accordingly, oxidative stress induced by HgCl$_2$ has been previously reported (Lund et al., 1991; Sener et al., 2007). As a consequence of lipid peroxidation biological membranes are affected causing cellular damage. In the present study, serum urea, creatinine, uric acid levels were significantly increased after 2 weeks mercuric chloride (0.5 mg kg$^{-1}$), showing insufficiency of renal function. Studies in animals have established that tubular injury plays a central role in the reduction of glomerular filtration rate in acute tubular necrosis. Two major tubular abnormalities could be involved in the decrease in glomerular function in mercuric chloride treated rats: Obstruction and backleak of glomerular Filtrate (Girardi and Elias, 1995). The alterations in glomerular function in mercuric chloride treated rats may also be secondary to ROS(reactive oxygen species), which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rat (Stohs and Bagchi, 1995; Zalups, 2000). Elevated serum level of the cytokine IL1, IL6 and TNFα demonstrated the severity of Hg induced system inflammatory response. Virgin olive oil as an antioxidant agent, ameliorated oxidative injury in the tissues and functional deteriorated. Both clinical event is perceived by tissue macrophages and monocytes, which in turn secrete cytokines such as
interleukine1, interleukine 6 and TNFα (Ziembia et al., 2005), indicating the role of this cytokine in this toxicity, while virgin olive oil depressed the IL1, IL6 and TNFα levels response. Thus, it seems likely that the alleviation of Hg induced oxidative tissue damage by virgin olive oil involves the suppression of a variety of pro-

inflammatory mediators produced by leukocytes and macrophages. The activity of GSH-Px and GST that can clear to protect the cells from being injured represents the competence of clearing free radicals from the organism. MDA content manifests the level of lipid peroxidation and then indirectly represents the level of damage of the cell of renal mitochondria. Evaluating from GSH, MDA levels and GSH-Px, GST activities in kidney of rats. Hg alone significantly decreased GSH level, GSH-Px and GST activities and increased MDA content along with histological damage in kidney.

Co-administration of virgin olive oil and Hg significantly increased GSH level and activities of GSH-Px and GST and decreased MDA content.

Several studies have demonstrated the ability of olive oil to inhibit oxidative stress in the liver through various mechanisms (Kyle et al., 1987). Moreover, we have shown that the oral supplementation of olive oil to rats administered ethanol chronically restored damage caused to the liver by inhibiting lipid peroxidation and improving enzymatic activities (Thabrew et al., 1987). The mechanism proposed to explain the positive effects of olive oil may be attributed to its richness in MUFA, mainly oleic acid which has different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria (Kasdallah-Grissa et al., 2008). Indeed, EVOO contains a considerable amount of oleuropein, hydroxytyrosol, tyrosol and caffeic acid which all have potent inhibition effects against ROS (Ochoa-Herrera et al., 2001; Owen et al., 2000). Hydroxytyrosol is highly effective against DNA damage by peroxynitrite in vitro (Deiana et al., 1999). Caffeic acid phenethyl ester and its related compounds limit the functional alterations of the isolated mouse brain and liver mitochondria submitted to in vitro anoxia-reoxygenation (Feng et al., 2008). Lipid peroxidation is the process of oxidative degradation of PUFA and its incidence in biological membranes resulting in impaired membrane function, structural integrity, decreased membrane fluidity and the inactivation of several membrane-bound enzymes (Gutteridge and Halliwell, 2000). Therefore, some particular attention was given to the liver’s fatty acid composition in rats used in the current experiment. In healthy humans, the short-term consumption of olive oil decreased serum oxidative stress (Weinbrenner et al., 2004) and their isolated lipoprotein fractions; LDL and HDL were shown to be enriched with oleic acid and resistant to oxidation (Aviram et al., 1993; Sola et al., 1997). Moreover, PUFAs are more susceptible to peroxidation resulting in MDA formation in mammalian tissues. In fact, because of their peculiar structure -that is the presence of one or more double bonds-UFA are more susceptible to free radical damage and thus could increase the susceptibility of LDL particles to oxidation. Most of the studies comparing the effects of a MUFA-rich diet with PUFAs-rich diet on LDL oxidation parameters have found a higher resistance of LDL particles to oxidation after the consumption of MUFA-rich diet (Estebauer et al., 1991; Aguileria et al., 2004). Finally, the health effects of dietary MUFA, including lower endothelial activation (Massaro and Caterina, 2002) and susceptibility of LDL to oxidation (Estebauer et al., 1991; Aguileria et al., 2004) are indeed to be considered. Nevertheless, it is also remarkable to establish the amount and quality of phenolic compounds in virgin olive oil.

5. CONCLUSION

It may be concluded that combined treatment of virgin olive oil has a preventive and protective effect on mercuric chloride induced oxidative stress. More-over, it protects from HgCl2 induced renal dysfunction and executes its modulatory role in mercury induced free radical production.

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