Original Article

Tyrosol and Olive Oil Ameliorate Sodium Arsenate-Induced Nephrotoxicity by Modulating of Oxidative Stress and Histological Changes in Mice

Abstract

Background: Sodium arsenate (Na 3As0 4, Sodium As) is an important toxic substance that leads to nephrotoxicity. Due to having bioactive molecules, such as polyphenols and tyrosol, olive oil plays a significant role in scavenging free radicals. This study aimed to investigate the effects of olive oil and tyrosol on As-induced nephrotoxicity. **Methods:** In our study, 42 adult male BALB/c mice were randomly divided into six groups: control (normal saline), olive oil (0.4 ml/d, gavage), tyrosol (5 mg/kg/d), Sodium As (15 mg/kg), olive oil + Sodium As, and tyrosol + Sodium As (olive oil and tyrosol received one hour before Sodium As). Drugs were administreted once daily for 30 consecutive days. On the 31st day of the study, oxidative stress parameters in kidney tissue, FRAP in plasma, renal function parameters in serum, and histopathological assays were performed. **Results:** Sodium As-induced renal damage as characterized by a significant increase of creatinine and BUN ($P < 0.001$) and histopathological changes. Also, Sodium As markedly altered oxidative stress biomarkers such as a significant increase in MDA ($P < 0.001$) and significantly decreased in FRAP and GSH ($P < 0.01$). Olive oil and tyrosol administration significantly improved the renal antioxidant defense system and decreased MDA concentration, markedly preserving the tissue structure and functional markers of kidney. However, these effects were more effective for tyrosol than olive oil. **Conclusions:** Our results suggest that olive oil and tyrosol can be used as a protective agent in preventing Sodium As-induced nephrotoxicity due to antioxidant property.

Keywords: *4-hydroxyphenylethanol, acute kidney injury, histology, olive oil, oxidative stress, sodium arsenate*

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Introduction

Sodium arsenate (Na 3As0 4, NaAs) can be found in food, soil, air and water.[1] Arsenic (As) is a toxic element that causes morbidity and mortality.[2] Exposure to As can cause infectious diseases,[3] cancer[4], and chronic diseases.[5] As, together with pro-inflammatory cytokines such as IL-6 and TNF-α, alters cell function and causes cell death.[6] Kidney is susceptible to As toxicity,[7] because it excretes As in urine.[8] Continuous exposure to As generates reactive oxygen species (ROS), which alters antioxidant parameters by increasing malondialdehyde (MDA), catalase (CAT), and decreasing glutathione (GSH) and GSH reductase levels in nephrons, causing nephrotoxicity.[9,10] Using exogenous antioxidants has been shown to reduce the damage caused by oxidative stress.[9,11]

Olive oil is a staple of the Mediterranean diet; diets high in olive oil are associated with low incidence of cardiovascular diseases, neurologic disorders,[12] and breast and colon cancer risk.[13] Polyphenols in olive oil are free radical scavengers that protect against oxidation of unsaturated fatty acids.[14] Olive oil exhibits anti-apoptotic, anti-inflammatory, and antioxidant properties.[15] The protective effect of olive oil against nephrotoxicity has been reported earlier in that it normalizes serum biochemical parameters and oxidative stress index, and thereby protects the kidney tissue.[15,16]

2-(4-Hydroxyphenyl) ethanol (Tyrosol) is a significant phenolic component of extra virgin olive oil[17]; it scavenges free radicals and inhibits lipid oxidation.[18] Tyrosol has been reported to be effective for preventing cerebral ischemia[19] healing muscle injury[20] and improving missing irradiated keratinocytes. Protective effect tyrosol has been reported for cerebral ischemia,[19] muscle injury[20], and irradiated

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keratinocytes.[21] Loru *et al*. (2009), however, reported that tyrosol does not protect against kidney cell damage caused by oxidative stress *in vitro*.[17]

We investigated whether olive oil or tyrosol, an active ingredient, exhibits antioxidant effects that protect against nephrotoxicity caused by NaAs in mice by biochemical and histological assessment.

Methods

Olive fruit was prepared from Roodbar city in the Gilan province of Iran. Tyrosol was purchased from Sigma (the United States), and NaAs was obtained from Merck Co (Germany).

Olive oil extraction and tyrosol content

Olive oil was extracted by mechanical procedures without using any solvent. In this way, we washed the olive, the core was separated, and then grind them. The milled olives were heated to two temperatures to give off their oil. Then, the oil was detached from the pulp by a filter. The olive extract was standardized based on tyrosol as the key active component**.** Olive oil and concentrations of tyrosol were injected on the HPLC column to draw a standard curve, then chromatograms were recorded at 280 nm. The amount of tyrosol in the olive oil was obtained using the peak surface area and standard curve.[22]

Animals and study design

Our experimental protocol was approved by the Institutional Animal Ethics Committee of the …. University Medical Sciences (ID: 2542).

Forty-two 25–30 g male BALB/c mice were was obtained from the Animal Research Center of …. University of Medical Sciences,…., Iran. Animals were housed in propylene cages in standard conditions (12 h light and 12 h dark), temperature (21 –22°C), and moisture (60 ± 5%) allowed to acclimate for a week before the study. Mice had free access to standard diet and water.

The mice were randomly divided into six groups and 7 animals in each group. Control group: the mice were administered normal saline, orraly, and Olive oil group: the mice were administered 0.4 ml olive oil, orraly daily for 30 days. Tyrosol group: the mice were administered 5 mg/kg tyrosol daily for 30 days, orraly. NaAs group: the mice were administered 15 mg/kg NaAs daily for 30 days, orraly Olive oil + NaAs group: the mice were adminstered olive oil and NaAs in the same doses as above. And the Tyrosol + NaAs group: the mice were administered tyrosol and NaAs in the same doses as above.

The dose of olive oil and NaAs was based on earlier reports[22,23] and the dose of tyrosol was based on a pilot study. NaAs was dissolved in distilled water. NaAs, olive oil, and tyrosol were administered by oral gavage using a sterile 26 gauge needle. Olive oil and tyrosol were administered 1 h before administering the NaAs.

Specimen collection

Animals were anesthetized with 50 mg/kg ketamine and 5 mg/kg xylazine intraperitoneally on day 31. Blood samples were collected from the heart, centrifuged at 3,000 × g for 15 min, and serum was stored at -20 ºC for evaluating serum biochemical markers. The kidneys were removed and one kidney was fixed with 10% buffered formalin for histological assay; the other kidney was frozen at -70 ºC to evaluate tissue biochemical biomarkers.

Serum biochemistry

Serum blood urea nitrogen (BUN) and creatinine were measured using kits: creatinine: BUN, 1-400-009 and creatinine, 1-400-029 (Pars Azmoon, Iran).

Oxidative stress analyses

Kidney GSH was measured using Ellman's reagent. Briefly, tissue homogenate (2.5%) was prepared in PBS (phosphate buffer saline) buffe (100 mM, pH 7.4) containing 0.02 M EDTA. We combined 2 ml homogenate, 1.8 ml water and 0.4 ml 50% TCA (trichloroacetic acid), to precipitate proteins. After centrifuging at 600 × g for 10 min, 2 ml of supernatant was transferred to a tube containing 100 ul DTNB (5,5′-dithiobis (2-nitrobenzoic acid) (0.01 M in ethanol), 0.01 M in ethanol, and 4 ml 0.4 M Tris-based buffer, pH 8.9, and the absorbance was recorded at 412 nm. GSH level was calculated from a standard curve plotted by different dilutions of standard GSH (0-0.6 Umol/mL prepared from Glutathione powder) (sigma, Germany).

Lipid peroxidation was measured in kidneys by estimating the level of MDA. 0.2-0.5 gr of tissue was homogenized in 1.15% KCl, and MDA was determined using TBA (Thiobarbituric acid) (sigma, Germany) reagent. The ferric reducing ability of plasma (FRAP assay) was measured using 2,4,6-tripyridyl-*s*-triazine (TPTZ) as described by Benzie and Strain.[24] Plasma antioxidant status was evaluated using the FRAP assay. At low pH, ferric tripyridyltriazine (Fe 3^{+}-TPTZ) complex is reduced to the ferrous (Fe 2^{+}) form, which is blue and its absorption was measured at 593 nm. The change in absorbance is directly proportional to the reducing power of the electron-donating antioxidants present in the plasma. FRAP was calculated using a standard curve of absorbanceplotted against the μM/l concentration of Fe (II) standard solution (0-100 uM/L Fe2+).

Histopathology

Kidneys were fixed with 10% formalin for microscopic evaluation. After fixation for 48 h, the specimens were

processed using a series of graded ethanol, clarified with xylene, and embedded in paraffin. Sections were cut at 5 μm using a microtome. To prepare a microscopic slide, sections were deparaffinized with xylene and rehydrated through decreasing concentrations of ethanol and mounted on glass slides. Sections were then stained with stained using the periodic acid-Schiff (PAS) procedure and examined by light microscopy (Olympus, Tokyo, Japan) at high magnification. Changes in renal tubules were investigated in 50 tubules for the extent of tubule and glomerulus damage, inflammatory cell infiltration, necrosis, edema, and desquamation. We used a semiquantitative scoring system: 0, no damage; 1, mild damage (<25%); 2, moderate damage (25–50%); 3, moderately severe damage (50–75%); 4, and severe damage (>75%).[25]

Statistical analysis

Data are means ± SD. The K-S test was used to evaluate the normality of the data. All comparisons were evaluated using one-way ANOVA and *post hoc* test of Tukey (Prism 7 software, USA. Values for $P \leq 0.05$ were considered as statistically significant.

Results

Tyrosol content of olive oil

Tyrosol was the principal phenolic compound in olive oil as determined by HPLC. Its concentration was determined to be 42.5 μg tyrosol/g olive oil.

Effects of olive oil and tyrosol on serum BUN and creatinine in NaAs-treated mice

Renal functional marker measurements are shown in Table 1. We found significant increases in serum BUN and creatinine levels in the group that received NaAs alone, compared to the control group ($P < 0.0001$). Olive oil and tyrosol administered to mice treated with NaAs significantly reduced BUN and creatinine**,** compared to the group that received only NaAs ($P < 0.05$ and 0.01, respectively. The improvement effected by tyrosol was greater than olive oil. In the groups treated with olive oil and tyrosol alone, BUN and creatinine levels were similar to the control group.

Table 1: Effect of olive oil and tyrosol on serum BUN and creatinine in NaAs-treated mice

Groups	BUN (mg/dl)	Creatinine (mg/dl)
Control	42.8±5.45	0.425±0.05
Olive oil	46.2±4.32	0.420±0.04
Tyrosol	39.8±3.7	0.417±0.04
NaAs	71.2±3.25 aaaa, bbbb, cccc	0.640±0.05 aaaa, bbbb, cccc
Olive oil + NaAs	61.3±7.31 aaaa, bbb, d	0.540±0.05 a,bb,d
Tyrosol + NaAs	57.5±5.96aa, cccc, dd	0.520±0.04c,dd

Data are means±SEM. a compared to control; b compared to olive oil, c compared to tyrosol; d compared to NaAs group

Effects of olive oil and tyrosol on oxidative stress markers in NaAs-treated mice

GSH levels reduced significantly in the renal tissues of the NaAs group as compared to the control group ($P < 0.001$) [Figure 1]. Administering olive oil and tyrosol to NaAs-treated mice increased the GSH level in comparison to the NaAs alone group, but the increase was statistically significant for the tyrosol + NaAs group ($P < 0.01$). Mice administered either olive oil or tyrosol alone had a slight increase in GSH level, compared to the control group, but it was not significant.

The TBARS content in NaAs-treated mice increased significantly compared to the control group. Co-administration of olive oil and tyrosol to NaAs-treated animals produced a significant decrease in the TBARS level in renal tissues compared to the NaAs-treated mice ($P < 0.01$).

The FRAP level in the plasma of NaAs group decreased significantly compared to the control group ($P < 0.001$). Administration of olive oil and tyrosol to the NaAs groups increased the FRAP level. This increase was more significant for tyrosol + NaAs than in olive oil + NaAs group ($P < 0.05$ and 0.01, respectively).

Effects of olive oil and tyrosol on histopathology in NaAs-treated mice

Photomicrographs of kidneys in all groups are presented in Figure 2. The control group exhibited normal renal structures [Figure 2a]. We found no histological change in the olive oil [Figure 2b] and tyrosol [Figure 2c] treated groups and it was similar to the control group. NaAs caused tubular swelling, tubular necrosis, epithelial cell degeneration, atrophy of glomeruli, congestion, thickened basement membrane, desquamation of epithelial cell, diffuse hemorrhage, and proteins penetrate the renal proximal tubules (luminal cast formation) [Figure 2d]. By contrast to the NaAs-treated group, co-administration of olive oil with NaAs and Tyrosol with NaAs clearly reduced all of pathological lesions [Figure 2e and f]. These findings are consistent with the results of serum biochemical biomarkers and oxidative stress measurements. Semiquantitative evaluation of renal injury is summarized in Figure 3.

Discussion

As is a toxic element that is widely distributed in the environment. NaAs-induced nephrotoxicity causes chronic or acute kidney damage.[26] We investigated the protective effects of olive oil and/or tyrosol against NaAs-induced nephrotoxicity. We found that NaAs-induced oxidative stress, functional damage, and histology. Olive oil and tyrosol administration in NaAs-treated mice improved renal injury and decreased oxidative stress markers.

Mitochondria are considered the primary target for arsenic toxicity. Inflammatory cytokines produce free radicals via

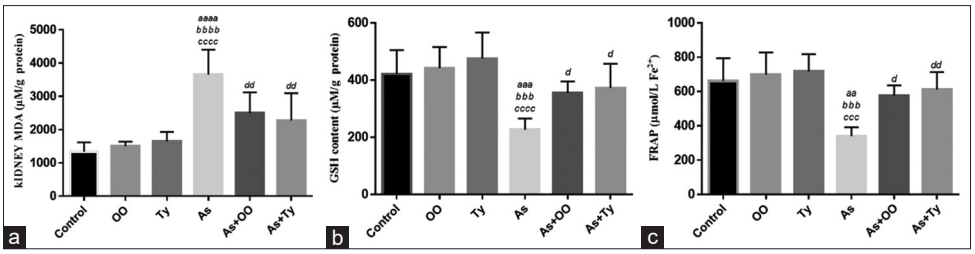

Figure 1: Effects of olive oil and tyrosol on oxidative stress. (a) MDA, (b) GSH, and (c) FRAP levels. Data are means ± SD. a compared to control group; b compared to. OO group; c compared to Ty; d compared to As. MDA, malondialdehyde; GSH, glutathione; FRAP, ferric reducing ability of plasma; OO, olive oil; Ty, tyrosol; As, sodium arsenate

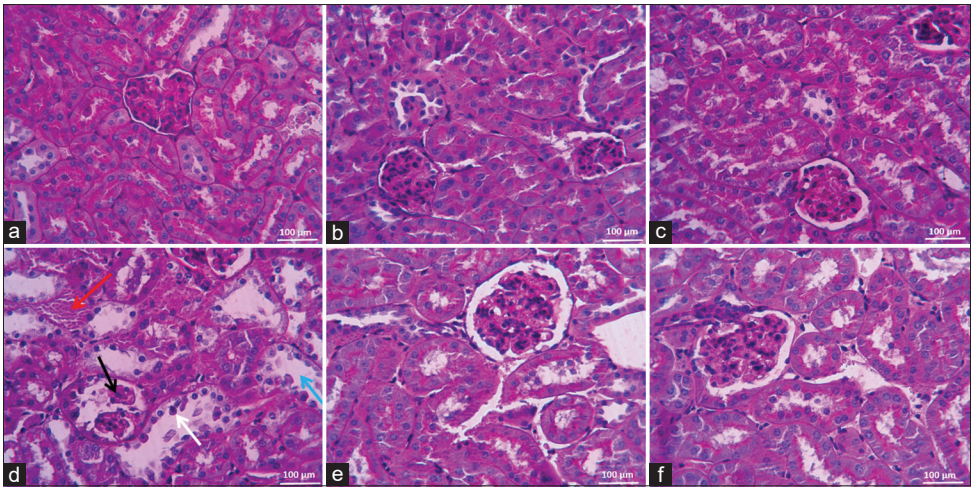

Figure 2: Renal tissue showing the effects of olive oil and tyrosol in NaAs-treated mice. (a) Control, (b) Olive oil, (c) Tyrosol groups appear normal, (d) NaAs group exhibiting tubule dilation (white arrow), epithelial desquamation (white arrow), atrophy of glomeruli (blue arrow), hemorrhage (red arrow), (e and f) Olive oil and tyrosol in NaAs-treated mice exhibit improved histomorphology. PAS staining, ×400, scale bars = 100 µm

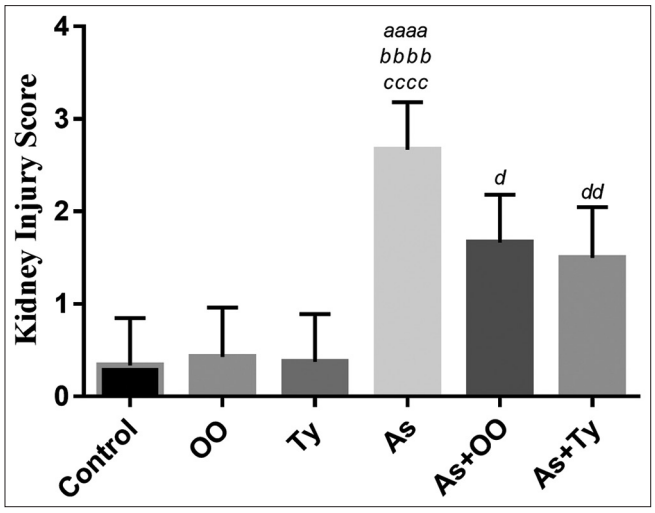

Figure 3: Kidney injury scores. Data are means ± SD. Administration of olive oil and tyrosol improved the score in NaAs-treated mice. *a* compared to control group; *b* compared to OO group; *c* compared to Ty; *d* compared to As. OO, olive oil; Ty, tyrosol; As, sodium arsenate

the mitochondrial respiratory chain.[23] NaAs causes renal injury by causing oxidative stress.[26,27] Also, As reduces the endogenous enzyme antioxidant defense system.[27] Also, administration of NaAs with the production of oxidative stress results in a significant decrease in the activities of brush border membrane enzymes in the renal epithelial cells. It also increases lipid oxidation and reduces antioxidant defense system activity.[28] We found that NaAs treatment increased MDA and decreased GSH and FRA*P* values in renal tissue and plasma, which is consistent with both increased ROS production and decreased antioxidant defense integrity. Disturbance of the normal oxidation state in cells can affect lipids, proteins, and DNA.[29] We found that the pro-oxidant effect of NaAs changed membrane integrity and fluidity in the cells via the generation of oxidative stress, especially MDA, as the index of lipid oxidation increased in the kidney.

Antioxidants can mitigate oxidative stress-induced damage. GSH maintains redox status in cells. ROS production and decreased GSH caused by As impairs mitochondrial function and activates pro-inflammatory signals and apoptosis. We found that oral administration of olive oil and/or tyrosol in NaAs-treated mice ameliorated non-enzymatic antioxidant levels. Zang also showed in a study that tyrosol is able to reduce FRA*P* value.[30] We reported earlier that olive oil improves As-induced hepatotoxicity by maintaining GSH, MDA, and FRAP levels.[22]

Owing to oxidative stress from the As exposure, antioxidant supplements may alleviate As-induced toxicity.

We found that antioxidants in olive oil protected the kidney from oxidative injury by scavenging ROS. Olive oil decreases lipid oxidation and enhances the antioxidant defense system because of its high phenolic content.[31,32] Phenolic compounds in olive oil prevent phospholipid oxidation-induced free radicals by binding to the phospholipid bilayer surfaces in the cell membrane.[33] Olive oil possesses anti-inflammatory, antimicrobial, antioxidant, anti-apoptotic, and anti-inflammatory properties.[14,34,35]

We found that olive oil administration in NaAs-treated mice decreased plasma creatinine and urea nitrogen concentrations, compared to the NaAs-treated group, which confirmed improved glomerular filtration rate. The protection afforded by olive oil may be attributed to the anti-oxidative polyphenols in olive oil. Tavafi *et al*. (2012) [36] found that olive leaf extract improved the levels of serum BUN and creatinine in gentamicin-induced nephrotoxicity. Additionally, Al-Attar *et al*. (2017)[37] reported that olive leaf extract ameliorated thioacetamide-induced nephrotoxicity. Polyphenols of olive oil inhibit nuclear factor κB to decrease inflammation.[34,38] The protective effects of olive oil are related to the main compounds of olive oil, which has antioxidant activity at a physiological concentration. [39,40] The biologic activity of these compounds has been demonstrated for physiological parameters including plasma lipoproteins, oxidative injury, inflammation, cellular function, antimicrobial effect and bone health. [41] Soni *et al*. (2018) reported that hydroxytyrosol is the main compound of olive oil with improvement on the antioxidant system efficiently decreases oxidative stress in NaAs-treated rat brain. Soni *et al*. (2017)[42] also reported that hydroxytyrosol can restore normal mitochondrial function inflicted by As.

We investigated the protective effect of 5 mg/kg tyrosol for the nephrotoxicity caused by NaAs and found significant improvements. Tyrosol and hydroxytyrosol are components of olive oil that are thought to be responsible for the pharmacological effects of olive oil.[17] We found that tyrosol is the main phenolic component of olive oil.[43] Tyrosol is excreted in the urine.[44] The protective effect of tyrosol against neuronal cell death and ethanol-induced oxidative stress of Hepg2 cells has been reported earlier.[45,46] Loru *et al*. (2009) reported that hydroxytyrosol maintains the integrity of the cell membranes in kidney cells during oxidative stress. Tyrosol, instead, does not display any protective effect.[17]

Serum urea and creatinine are renal functional markers that increase by kidney failure.[47] Creatinine is filtered by glomeruli and excreted into the urine. Serum creatinine concentration is a stronger marker than urea during the early phase of kidney disease.[48] The concentration of urea in serum increases the following damage to kidney tissues.[36] We found that administration of 15 mg/kg/day NaAs increased serum creatinine and BUN significantly, which is consistent with an earlier report.[49] We found that co-administration of olive oil with NaAs mitigated serum creatinine that shows the alleviation of glomerular function by olive oil administration. These findings are due to improvement of tubule and glomerular function produced by olive oil in As-induced kidney toxicity.

The kidney is vulnerable to ROS because it is rich in unsaturated fatty acids.[17] Changes in renal structure in NaAs-treated mice were consistent with our biochemical findings. These changes were characterized by glomerular atrophy, tubular necrosis, glomerular space dilation, inflammatory cell infiltration, hemorrhage, and renal tubule dilation. Histopathologic findings are likely due to accumulation of free radicals and increased oxidative stress in the renal tissue of mice treated with NaAs. In addition, cellular damage and necrosis stimulate inflammatory processes, which in turn exacerbates leukocyte migration to the affected site.[27] Reduction of cell necrosis and exfoliation of epithelial cells into the lumen of the renal tubule, together with reduced inflammatory processes and leukocyte infiltration, improved the glomerular filtration rate, which improved kidney function.

We confirmed the nephroprotective potential of olive oil and tyrosol against chronic kidney failure induced by NaAs. Administration of olive oil and/or tyrosol decreased lipid oxidation and increased renal GSH and plasma FRAP level and histopathology due to their antioxidant properties. Olive oil and tyrosol appears to be a natural dietary source for reducing chronic kidney damage caused by As.

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Conflicts of interest

There are no conflicts of interest.

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