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Effect of curcumin on kidney histopathological changes, lipid peroxidation and total antioxidant capacity of serum in sodium arsenite-treated mice

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ABSTRACT

Sodium arsenite is an environmental pollutant with the ability to generate free radicals and curcumin acts as a potent antioxidant. This study investigates the effect of curcumin on kidney histopathology, lipid peroxidation and antioxidant capacity of serum in the mice treated with sodium arsenite. Adult male mice were divided into four groups: control, sodium arsenite, curcumin and curcumin + sodium arsenite. The treatments were delivered for 5 weeks. After the treatment period, blood samples were collected and the concentrations of malondialdehyde (MDA) and total antioxidant capacity of serum were determined. Left kidney was dissected, weighed and used for histopathological and histomorphometrical studies. Sodium arsenite-treated mice showed a significant decrease in the diameter of glomerulus and proximal tubule, glomerular area, total antioxidant capacity of serum as well as a significant increase in serum concentration of MDA compared to the control group. However, no significant difference was found in kidney weight, area and diameter of Bowman's capsule as well as the diameter of distal tubule in mice treated with sodium arsenite compared to the control. In curcumin + sodium arsenite group, curcumin significantly reversed the adverse effects of sodium arsenite on the diameter of glomerulus and proximal tubule, glomerular area, total antioxidant capacity of serum and serum concentration of MDA compared to the sodium arsenite group. The application of curcumin alone significantly increased the total antioxidant capacity of serum compared to the control. Curcumin compensated the adverse effects of sodium arsenite on kidney tissue, lipid peroxidation and total antioxidant capacity of serum.

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1. Introduction

Environmental pollution is one of the greatest threats to human health. Human activity releases toxic heavy metals such as arsenic. Arsenic mobilization in the environment is enhanced by microorganisms but human intervention also affects the natural equilibrium of the ecosystems (Akter et al., 2005). Arsenic compounds are used to make special glass types, wood preservatives, herbicides, insecticides (Kannan and Flora, 2004) as well as medicines for the treatment of blood cancer (Hu et al., 2005). The main source of arsenic exposure is via the use of arsenic-contaminated foods and water that endangers health (Ayotte et al., 2003; Karagas et al., 2002). Arsenic poisoning has adverse effects on human health (Ahmad et al., 2001; Rossman, 2003) and damages all the target organs including kidney (Blanca et al., 2007). Decrease in tubular volume (Rubatto Birri et al., 2010) and lumen (Ferzand et al., 2008), the shrinkage of the glomerulus (Roy and Bhattacharya, 2006), increase in the Bowman's space, acute tubular and glomerular degeneration (Ferzand et al., 2008) and increase in blood creatinine and urea (Rizwan et al., 2014) are some kidney complications associated with arsenic exposure. Several lines of evidence have shown that arsenic exerts its toxicity through reactive oxygen species (ROS) and generation of free radicals (Gurr et al., 2003; Shi et al., 2004). Arsenic is able to induce lipid peroxidation in the membranes (Gupta and Flora, 2005), leading to apoptosis in a wide variety of cells (Das et al., 2009). Therefore, the application of antioxidants, particularly the antioxidants which are derived from medical plants, can be a possible strategy for protecting cellular damages against arsenic toxicity. Antioxidants are known as free radical scavenger and reduce the impact of the free radicals created by oxidative stress (Bengmark, 2006).

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Curcumin is a polyphenol compound derived from the tumeric with powerful anti inflammatory and antioxidant properties (El-Wakf et al., 2011). In addition, the presence of methoxy groups on the phenyl ring increases curcumin activity (Priyadarsini, 2013). Curcumin is shown to inhibit ROS formation and scavenge free radicals in pathological conditions, resulting in the protection of vital cellular components such as lipids, proteins and DNA (El-Wakf et al., 2011).

Previous studies have shown the adverse effects of sodium arsenite on kidney. To our knowledge, however, no study has examined the effect of curcumin on sodium arsenite mediated toxicity in kidney histopathological changes and serum antioxidant capacity of adult mice. The present study was therefore conducted to investigate the effect of curcumin on kidney histopathology and histomorphometry, lipid peroxidation index and total antioxidant capacity of serum in adult mice treated by sodium arsenite.

2. Material and methods

2.1. Animals and treatments

In this experimental study adult male NMRI mice (32 ± 5 gr) were purchased from Pasture's Institute, Tehran, Iran. The animals were housed in plastic cages at 12-h light/dark cycle, 24 ± 2 °C with water and food available *ad libitum*. The mice were divided into four groups (n = 6 for each group): control, sodium arsenite (5 mg/kg, Sigma, USA), curcumin (100 mg/kg, Sigma, USA) and curcumin + sodium arsenite. The treatments were delivered by intraperitoneal injection for five weeks (Chinoy et al., 2004). The experiments were approved by the local ethical committee at Arak University. Sodium arsenite and curcumin were dissolved in distilled water and dimethyl sulfoxide (DMSO) respectively. Based on the solvants, two control groups were selected; distilled water and DMSO. Since no significant difference was found between the results of the controls, distilled water data was considered as control group. At the end of the treatments, the animals were weighed and anesthetized. Blood samples were collected from the heart and centrifuged, and serum samples were stored at −80 °C for biochemical analysis. Left kidney was dissected, decapsulated, weighed and fixed for the histopathological and histomorphometrical studies.

2.2. Tissue preparation for histopathological and histomorphometrical evaluation

The kidneys were fixed in Bouin's fixative for 36 h. They were subsequently dehydrated, embedded in paraffin, sectioned at 5 μm and stained via Heidenhain's Azan method (Mehranjani et al., 2009). The sections were then examined and photographed under an optical microscope. Motic Image 2000 Software was used to measure the area and the diameter of glomerulus, Bowman's capsule as well as distal and proximal tubules.

2.3. Assessment of serum malondialdehyde

Malondialdehyde (MDA) is an end-product of lipid peroxidation during oxidative stress and is frequently used as an indicator of lipid peroxidation. The amount of serum MDA was measured using the thiobarbituric acid (TBA) assay according to the method described by Turki and Moayad Naji (Turki and Moayad Naji, 2011). In brief, 1000 μl of trichloroacetic acid, thiobarbituric acid and hydrochloric acidreagent (TCA15% w/v, TBA 0.375% w/v and HCl0.25 N) was added to 500 μl of serum sample and heated for 15 min in boiling water bath. The samples were then cooled and centrifuged at 1000*g* for 10 min. The absorbance of the supernatant was determined using a spectrophotometer at 535 nm. The MDA concentration was calculated by its molar extinction coefficient $1.56 \times 10^5 M^{-1} cm^{-1}$ and expressed as nmol/ml.

Table 1
Kidney weight in mice treated with sodium arsenite and curcumin.

Weight (g)	Control	Curcumin	Sodium arsenite	Curcumin + Sodiumarsenite
Kidney	0.232 ± 0.02	0.245 ± 0.01	0.215 ± 0.01	0.231 ± 0.02

Mean ± SD, n = 6 per group. ANOVA, Tukey's test, $p < 0.05$.

2.4. Assessment of total antioxidant capacity of serum

The ferric reducing antioxidant power assay (FRAP) is a routine method for estimating total antioxidant capacity. The principle of this method is based on the reduction of Fe^{3+} to Fe^{2+}due to the action of antioxidants. Interaction of $Fe^{2+}$with 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) provides a maximum absorption at 593 nm (Emin et al., 2010). Briefly, 900 μl of freshly prepared and pre-tempered (37 °C) FRAP reagent (300 mM acetate buffer, pH 3.6 with 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in ratio10:1:1 respectively) was added to 100 μl of serum (previously diluted 1:1 with distilled water) which was then incubated for 4 min at 37 °C. The absorbance was determined at 593 nm by a spectrophotometer. Different concentrations of $FeSO_4 \cdot 7H_2O$ were used for the preparation of standard curve preparation. The total antioxidant capacity of the samples was then calculated using regression equation obtained from the standard curve and expressed in mmol/l.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation (SD) for six animals per group. One-way analysis of variance (ANOVA) followed by Tukey's test was used to assess the statistical significance of data. $p < 0.05$ was considered significant.

3. Results

3.1. Kidney weight

No significant difference was found in kidney weight among the four groups ($p > 0.05$) (Table 1).

3.2. Kidney histopathology

The control and curcumin groups (Fig. 1a–d respectively) displayed an expected structure of renal tubules, glomeruli and Bowman's capsule. Shrinkage of glomerulus, increase in the Bowman's space, vacuolation of tubular epithelium and acute tubular and glomerular degeneration were observed in the sodium arsenite group (Fig. 1b). In addition, degenerated epithelial cells were extruded into tubular lumen in this group. Curcumin compensated for the adverse effect of sodium arsenite in the curcumin + sodium arsenite group compared to the sodium arsenite group (Fig. 1c).

3.3. Kidney histomorphometry

The histomorphometrical analysis of the kidney (Table 2) showed a significant decrease in the diameter of glomerulus and proximal tubule ($p < 0.001$) as well as glomerular area ($p < 0.01$) in the sodium arsenite group compared to the control. Curcumin significantly reversed the adverse effect of sodium arsenite on the diameter of glomerulus and proximal tubule ($p < 0.001$) as well as glomerular area ($p < 0.05$) in the curcumin + sodium arsenite group

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