Vitamin C Ameliorates Acrylamide-Induced Nephrotoxicity and Improves the Biochemical Parameters in Rats

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Abstract

Background & Objective: Acrylamide is a highly soluble, widely-produced industrial and cytotoxic material. Some substances with antioxidant properties can ameliorate the deleterious effect of acrylamide. Vitamin C is necessary for the normal functioning of all cells and scavenging the free radicals due to the antioxidant properties. The present study was aimed to evaluate the effect of vitamin C on the biochemical parameters and histological changes in the kidney tissue damage induced by acrylamide in rats.

Materials & Methods: Forty rats were randomly divided into four groups (n=10): 1) the control group, 2) acrylamide group, 3) vitamin C group, and 4) acrylamide + vitamin C group. Histopathologic assessment (by Hematoxylin and Eosin (H&E) staining of the kidney tissue) was performed and biochemical parameters (serum malondialdehyde, total antioxidant capacity (TAC), urea, and creatinine) were measured.

Results: There was a significant enhancement in the serum urea, creatinine, and malondialdehyde levels in the acrylamide group compared to the other groups (P < 0.001). Serum TAC increased in the vitamin C group compared to the acrylamide + vitamin C and acrylamide groups (P ≤ 0.001).

Conclusion: The present study showed that chronic consumption of acrylamide can lead to pathological changes in the kidney tissue as well as unfavorable alteration in serum urea, creatinine, TAC, and MDA levels. Concurrent vitamin C consumption had a significant preventive effect on the aforementioned parameters. Therefore, vitamin C can play a protective and antioxidant role in decreasing the toxic effects of acrylamide in rat kidneys.

Keywords: Acrylamide; Biochemistry parameters; Histology; Kidney; Vitamin C

Introduction

Acrylamide (2-propenamide), an odorless white crystalline material, was discovered in in Germany in 1893 by Moureu. Acrylamide has a melting point of 84.5°C that is stable at room temperature, it is rapidly polymerized upon melting (1). Acrylamide is present in highly-heated foods such as fried potatoes, spaghetti, and bread (2). Exposure to acrylamide occurs mainly through oral intake, skin contact, and inhalation. The main mechanism for acrylamide formation in foods is related to the Maillard reaction (3).

After consumption, monomers of acrylamide are easily absorbed from the digestive tract, enter the blood circulation, spread to different organs, react with hemoglobin, DNA, and some cellular enzymes, and produce various toxic effects (4). It can pass through the placenta and be transmitted to the fetus (5). Glomerular changes, infiltration of inflammatory cells, and degenerative vascular changes have been observed in the kidney which is the main organ eliminating acrylamide and its metabolites (6). Acrylamide induces oxidative stress, resulting in the generation of reactive oxygen species (ROS) (7) which play a critical role in the initiation and progression of fibrotic diseases (8) and can damage some organs and tissues such as the liver and kidney (2, 4, 8-10).

The antioxidant system confronts the damage resulted from free radicals via preventing the formation of free radicals, restoring the damages caused by radicals, increasing the excretion of damaged molecules, and minimizing cellular mutation. Enzymatic antioxidant defense systems include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, and non-enzymatic substances including antioxidant compounds in foods (e.g. vitamins E, C, and A) (11). Vitamin C or ascorbic acid is necessary for growth, development and the normal functioning of all cells including collagen biosynthesis, immune system, and wound healing due to the presence
of the antioxidant properties (12). In addition to scavenging the free radicals, vitamin C causes the re-entry of other antioxidants such as vitamin E and urate into the cycle.

In recent years, much attention has been paid to the presence of acrylamide in foods. The increasing human exposure to acrylamide has led the researchers to study the harmful effects of acrylamide intake as well as the use of the antioxidants in order to prevent oxidative tissue damage. The present study aimed to evaluate the effect of vitamin C on the biochemical parameters and histological changes in the kidney tissue damage induced by acrylamide in rats.

Materials and Methods

Animals

The present experimental study was carried out on 40 male rats which were kept from birth under standard conditions (free access to food and water, the controlled temperature at 21 ± 3 °C, and 12 hours of light/12 hours of darkness) in the animal house of Shahid Sadoughi University of medical sciences in Yazd, Iran.

Animal Ethics

All ethical principles of laboratory animal procedures were in accordance with those approved by the Ethics Committee of Payame Noor University (Ethics committee code: IR.PNU.REC.1397.042).

Experimental Design

Forty rats were randomly divided into four groups with 10 rats in each group. The first group received distilled water (normal group), daily, the second group received 20 mg/kg/day acrylamide (Merck, Germany) (AA group) (8, 13, 14), the third group received 200 mg/kg/day vitamin C (Darupakhsh Co., Iran) (vitamin C group) (15) and the fourth group received 20 mg/kg/day acrylamide plus 200 mg/kg vitamin C (AA + vitamin C group). The animals weighing 60-80 g were fed by the oral gavage from infancy for 2 months at a specific hour. Six hours after receiving the last dose of treatments, the animals were generally anesthetized via intraperitoneal injection of xylazine 5 mg/kg plus ketamine 50 mg/kg (Alfasan Co, The Netherlands), and the blood samples were taken from the right atrium for biochemical tests. The biochemical and antioxidant assessments were carried out in duplicate. The kidney was also removed and placed in buffered 10% formalin fixative for evaluation of histologic changes. The assessors, involved in the biochemical, antioxidant, and histological assessment were blinded to the treatment allocation.

Biochemical and Antioxidant Tests

Serum levels of urea and creatinine were measured using enzymatic and Jaffe method by commercial kits (Man Company, Iran), respectively.

The reaction with thiobarbituric acid (TBA) is the basis for malondialdehyde measurement (16). MDA reacts with TBA and the resulting red color has the highest light absorbance at 532 nm. In brief, 0.1 mL of serum was mixed with 0.375 mL of 20% acetic acid and 0.375 mL of 0.6% TBA solution which was heated for 60 minutes in a boiling water bath. The solution was then placed in the cold water for 10 minutes, to which 1.25 mL of normal butanal/pyridine with 1:15 ratio was added and then centrifuged for 5 minutes at about 2000 rpm. Then, the butanal phase absorption was assayed at 532 nm. The MDA concentration was reported in µmol/mL (7).

Total antioxidant capacity (TAC) was estimated via the reduction of diphenyl-picrylhydrazyl (DPPH). To do this, 0.0039 g of DPPH powder was mixed with 10 mL of methanol, the solution was mixed by a vortex, and 1 mL of it was added to 9 mL of methanol. Then 20 µL of sample and 380 µL of phosphate buffer (pH=7.4) were mixed with 400 µL of DPPH methanol solution by a vortex. The solution was incubated for half an hour at room temperature. Then, 100 µL of the working solution was added to the 96-well microplate and then measured at 520 nm by enzyme-linked immunosorbent assay (ELISA) reader. The concentration of DPPH scavenging capacity was calculated according to the following formula (7):

\[
DPPH \text{ reduced (\%)} = \frac{[A-A_x]}{A_x} \times 100
\]

\[A = \text{Absorbance of (DPPH + plasma), } A_x = \text{Absorbance of (DPPH + methanol as blank)}\]

Histological Preparation and Techniques

An assessor who was blinded to the allocation selected the 5 rats in each group for histologic measurement, randomly. The kidney's rats were fixed by the formalin solution and sectioned and passed through the tissue processor (Lepsaw Model 2500, US) followed by paraffin embedding (Did Sabr paraffin dispenser, model DS-4LM, Iran). Three sections of 5-7- micrometer-thickness were made for each specimen by a microtome (Leitz 1512, Germany) (17). Hematoxylin and eosin-stained (H&E) slides were imaged (Olympus, model CH-50, Japan) and studied by a blinded pathologist.

Statistical Analysis

The comparison of the biochemical parameters was analyzed using one-way ANOVA with Tukey post-test, and shown as mean ± standard deviation (SD). SPSS statistical software package, version 16.0 (SPSS, Inc, Chicago, Illinois, USA), was applied for statistical analyses. The significance level was considered at P ≤ 0.05.

Results

The biochemical parameters were shown in Table 1 and figure S.1-4 (supplementary file). The mean serum urea (mg/dL) demonstrated a significant increase in the AA group compared to the normal group (48.1 ± 1.2 vs. 38 ± 0.6, P ≤ 0.001). The mean serum urea in the vitamin C group was significantly lower than the AA group (35.5 ± 1.1 vs. 48.1 ± 1.2, P ≤ 0.001). In the AA + vitamin C group, serum urea was significantly lower than the AA group (38.6 ± 0.9 vs. 48.1 ± 1.2, P ≤ 0.001). Furthermore, no significant difference was found between the vitamin
Effect of vitamin C on acrylamide-induced nephrotoxicity

C, AA + vitamin C, and normal groups (P > 0.05) (Figure S.1. supplementary file).

The mean serum creatinine levels (mg/dL) were significantly higher in the AA group compared to the normal group (1.5 ± 0.1 vs. 0.7 ± 0.04, P ≤ 0.001). A decrease in serum creatinine levels was found in the AA + vitamin C group (0.9 ± 0.04) and the vitamin C group (0.7 ± 0.04) compared to the AA group (1.5 ± 0.1), (P < 0.001). There was no significant difference between the AA + vitamin C, normal, and vitamin C groups (P > 0.05) (Figure S.1. supplementary file).

The mean serum creatinine levels (mg/dL) were significantly higher in the AA group compared to the normal group (1.5 ± 0.1 vs. 0.7 ± 0.04, P ≤ 0.001). A decrease in serum creatinine levels was found in the AA + vitamin C group (0.9 ± 0.04) and the vitamin C group (0.7 ± 0.04) compared to the AA group (1.5 ± 0.1), (P < 0.001). There was no significant difference between the AA + vitamin C, normal, and vitamin C groups (P > 0.05) (Figure S.1. supplementary file).

The results of the serum TAC (%) as a marker of antioxidant capacity were revealed in Table 1. A significant reduction was found in the AA group in comparison with the normal group (25.81 ± 2.3 vs. 46.78 ± 2.3, P ≤ 0.001). In comparison to the AA group, the AA + vitamin C group had no significant enhancement (34.46 ± 3.03 vs. 25.81 ± 2.3, P = 0.14). In addition, the serum TAC (%) in the vitamin C group was significantly higher than the AA group (54.52 ± 3.2 vs 25.81 ± 2.3) and the AA + vitamin C groups, (P ≤ 0.001). Moreover, there was no significant difference between the vitamin C and normal groups (P= 0.21) (Figure S.3. supplementary file).

MDA (µmol/mL), as a marker of oxidative stress and lipid peroxidation, was markedly higher in the AA group compared to all other groups (P ≤ 0.001). A significant increase was observed in the AA group compared with both the normal (0.3 ± 0.04 vs. 0.16 ± 0.007, P = 0.002) and the AA + vitamin C group (0.3 ± 0.04 vs. 0.18 ± 0.034, P = 0.004). The vitamin C group revealed a significant decrease in MDA compared to the AA group (0.09 ± 0.005 vs. 0.3 ± 0.04, P ≤ 0.001). There were no significant differences between other groups (P > 0.05) (Figure S.4. supplementary file).

### Table 1. The comparison of the biochemical parameters between four groups.

<table>
<thead>
<tr>
<th>Biochemistry parameters</th>
<th>Group</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal (n=10)</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>38 ± 0.6^a</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 ± 0.04^a</td>
<td></td>
</tr>
<tr>
<td>TAC (%)</td>
<td>46.78 ± 2.3^a</td>
<td></td>
</tr>
<tr>
<td>MDA (µmol/mL)</td>
<td>0.16 ± 0.007^a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acrylamide (n=10)</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>48.1 ± 1.2^abc</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.5 ± 0.1^abc</td>
<td></td>
</tr>
<tr>
<td>TAC (%)</td>
<td>15.5 ± 1.1^b</td>
<td></td>
</tr>
<tr>
<td>MDA (µmol/mL)</td>
<td>0.3 ± 0.04^abc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin C (n=10)</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>35.5 ± 1.1^b</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 ± 0.04^b</td>
<td></td>
</tr>
<tr>
<td>TAC (%)</td>
<td>54.52 ± 3.2^c</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>MDA (µmol/mL)</td>
<td>0.09 ± 0.005^b</td>
<td></td>
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<tr>
<td></td>
<td>Acrylamide+ Vitamin C  (n=10)</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>38.6 ± 0.9^c</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 ± 0.04^c</td>
<td></td>
</tr>
<tr>
<td>TAC (%)</td>
<td>34.46 ± 3.03^c</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>MDA (µmol/mL)</td>
<td>0.18 ± 0.034^c</td>
<td></td>
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</tbody>
</table>

MDA: malondialdehyde; TAC: Total antioxidant capacity.
Data were presented as mean ± standard deviation.
P-value was calculated for the comparison variables between four groups using one-way analysis of variance (one-way ANOVA).
*Pairwise comparisons were performed by post hoc (LSD) analysis.

The renal histological assessment was depicted in Figure 1.A to D. As projected in Figure 1.A, in the normal, AA, AA + Vitamin C, and Vitamin C groups, respectively. No clear pathologic finding was observed in the normal group. There were normal glomeruli (green arrow) and tubules (blue arrow) in the normal group (Figure 1.A). The renal tissue changes in the AA group was represented in Figure 1.B, the glomerular degeneration/atrophy (green arrows), the expansion of Bowman's capsule (asterisk), and the destruction of the epithelial cells were observed in the convoluted tubules (blue arrows). The numbers of glomeruli which were counted under high power fields (400X), were lower than those of the other groups. Moreover, hyperemia was observed in the renal vessels (yellow arrow). The proximal and distal-tubular necrosis and inflammation and infiltration of inflammatory cells were seen in the kidney cortex. In addition, the thickening and fibrosis of the kidney capsule were found in the AA group. In the AA + vitamin C group, the degenerative changes were less than the AA group (Figure 1.C). The glomeruli (green arrow) were larger than those in the AA group, and the proximal-tubular degeneration was not observed (blue arrow). Figure 1.D showed the histological assessment in the vitamin C group. Although the normal glomeruli (green arrow) without inflammation were observed in the interstitial tissue (blue arrow) in the vitamin C group, mild hyperemia was depicted in the renal parenchyma (yellow arrow).
Discussion

The findings of the present study showed a significant increase in serum urea, creatinine, and MDA levels in the AA group compared to the other groups with no remarkable difference between the normal, vitamin C, and AA + vitamin C groups. The serum TAC levels showed a clear increase in the vitamin C and normal groups compared to AA and AA + vitamin C groups. Serum MDA levels were significantly lower in the vitamin C group than the other groups. The evaluation of the renal tissue changes showed a considerable difference between the control group with the other groups. In the AA + vitamin C group, pathological changes were lower in comparison with the AA group, which can justify the protective and antioxidant role of vitamin C in preventing the toxic effects of acrylamide. There was no report on the adverse effects on the kidney function. Based on a comprehensive review of the literature (8, 13-15), the dose of vitamin C or acrylamide which was selected caused no severe adverse effects.

Acrylamide can cause the renal and hepatic tissue damage (8, 10, 18-20), the unfavorable alterations on serum urea and creatinine levels in rats (8, 18). The protective effects of vitamin E in acrylamide-induced renal toxicity in rats had been addressed in the study by Ated et al (8). A significant decrease in the serum creatinine and urea levels was shown in the acrylamide and vitamin E groups compared to the acrylamide group which is similar to our study on the protective effect of vitamin C (8). Salman et al., (2020) showed that oral ascorbic acid can improve acrylamide-induced hepatotoxicity in rats (20). The results of another study (2011) showed that the treatment with natural products containing cocoa in human cell cultures can decrease acrylamide-induced ROS production and prevent apoptosis (21). Acrylamide exposure in the animals resulted in the DNA damage, inflammation, necrosis, and apoptosis in the liver (4).

Moreover, Mahmood et al., (2015) (10) investigated the effects of different doses of acrylamide in Wistar
rats. In support of the findings of our study, Mahmood et al., showed more degenerative changes at doses of 10 and 30 mg of acrylamide per kg (mg/kg) than 2 mg/kg on the kidney and liver tissue. They found no significant difference between serum urea and creatinine levels in the acrylamide group (with a daily dose of 2 mg/kg of body weight) in comparison with the normal group (receiving distilled water). Contrary to expectations, they illustrated a significant decrease in serum urea and creatinine levels in rats that received doses of 10 (moderate dose) or 30 (high dose) mg/kg of body weight from the normal group. Their results were in contrast with the findings of the present study and other studies (8, 18). However, they reported that a moderate or high dose leads to a decrease of about 25% of nephron function, and for the changes in serum levels, the function of nephrons should reach less than 30% (10), but it seems that there is no scientific justification for lowering serum urea and creatinine levels.

The deleterious effects of acrylamide were addressed on histological changes, blood and enzyme parameters in rats. The researchers found that acrylamide can decrease serum antioxidant (serum vitamin C), increase MDA, and also cause histopathological alterations in the kidney (22).

Alturfan et al., showed that resveratrol as an antioxidant could promote the enhanced MDA levels, reduce GSH levels, and oxidative stress damage in the kidney induced by acrylamide. They suggested that the resveratrol supplementation can be useful for reducing acrylamide toxicity (23).

Moreover, Rajeh et al., found that the combined administration of vitamin E and 5-amino salicylic acid reversed the toxic effects of acrylamide on renal tubules (19). The protective effect of vitamin E on acrylamide-induced liver tissue damages was shown by Siahkooohi (24). His findings for the different antioxidants (vitamin E and 5-Aminosalicylic acid co-supplementation, resveratrol, vitamin E, and cocoa) (19, 21, 23, 24) were similar to the present results for vitamin C.

However, the oxidative stress which can lead to an imbalance in antioxidant capacity may be considered as the main factor for deleterious effects of acrylamide (25). Acrylamide may exert its effects by binding the sulhydryl groups to the proteins and enzymes resulting in DNA repair and cell dysfunction (26), decreasing the antioxidant storages, mitochondrial dysfunction, increasing the inflammatory markers (interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α)), and apoptosis (21, 27) by increasing caspase-3 (27) that leads to toxicity in the nerve system (1), and dysfunction of the liver (4, 10, 18), kidney (18, 22, 23), and reproductive process, and the carcinogenic effects on different organs (1, 25, 27).

To date, the effects of vitamin C have not been evaluated on acrylamide-induced nephrotoxicity. On the other hand, several mechanisms have illustrated the protective effects of other antioxidants regardless of antioxidant power including blocking acrylamide formation emanating from the increasing or decreasing reaction between the substrates in the Maillard reaction (28), scavenging free radical activity (28, 29), neurogen-erative activities, detoxification, inhibition of MAO activity, and acetyl-cholinesterase (27). It is noteworthy that some antioxidants with weak activity such as naringenin (30) can be more effective than the potent antioxidants including curcumin (31).

The strengths of the present study were 1) the long-term follow-up (2 months) of acrylamide-induced toxicity at the moderate dose and the protective effect of vitamin C in rats while the duration of similar previous studies were 5 (19), 10 (23), and 35 days (24); and 2) the large sample size in comparison to other studies (19, 23, 24).

The main limitation of this study and some others (8, 13, 14) was the use of a single-dose acrylamide and vitamin C.

Conclusion

In conclusion, the present study showed that chronic consumption of acrylamide can lead to renal dysfunction as indicated by the elevated serum urea and creatinine levels, as well as imbalance in serum antioxidant (TAC) and oxidation (MDA) levels. Concurrent vitamin C consumption had a significant preventive effect on the aforementioned parameters, in addition to the prevention of renal tissue degeneration. It seems to be necessary to design the studies with the prescription of different doses of vitamin C and acrylamide and also, the evaluation of the molecular and cellular mechanisms of vitamin C against acrylamide.

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Conflict of Interest

The authors declare no competing interest.

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