Astaxanthin; a Promising Protector Against Gentamicin-Induced Nephrotoxicity in Rats

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Abstract: Gentamicin is an aminoglycoside antibiotic widely used against infections caused by Gram-negative microorganisms. Nephrotoxicity is the main limitation to its therapeutic use. The objective of this study was to evaluate the potential protective effect of astaxanthin on the renal damage generated by gentamicin in rats, in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic applications. Daily oral administration of astaxanthin at a concentration of 50 mg/kg for 15 days to gentamicin (80 mg/kg.h.w) treated rats showed a significant decrease (p<0.05) in plasma creatinine, urea, TNF-α as well as plasma and renal MDA and HP. The treatment also resulted in a significant increase in hemoglobin, plasma sodium, potassium and TAS as well as renal total protein, GSH, Pr-SHs, G6PD, SOD, GPx, CAT and GR levels. The histological examinations of renal tissues in this study revealed damage and glomerular infiltration in gentamicin treated rats. The presented data suggest that astaxanthin has a significant prophylactic action against gentamicin-induced nephrotoxicity in rats. The effect was more pronounced in case of astaxanthin pre-treatment compared with administration of astaxanthin post-treatment. Taken together, astaxanthin has a potential as a protective and therapeutic agent for nephrotoxicity and deserves clinical trial in the near future as an adjuvant therapy in patients treated with gentamicin.

Keywords: Nephrotoxicity, gentamicin, astaxanthin, oxidative stress biomarkers and antioxidant.

INTRODUCTION

Gentamicin (GM) is probably the most commonly used and studied antibiotic of all aminoglycosides, however, it causes severe renal toxicity [1, 2]. Many studies have postulated that renal inflammation is involved in the damage [3] as well as necrosis of renal tubular epithelial cells [4]. Gentamicin causes damage acting through free radicals [5]. Gentamicin increases urinary excretion of sodium and potassium mostly due to the chronic administration of the drug [6]. Serum creatinine concentration is a more potent indicator than the urea in the early stadium of kidney disease. Urea concentrations start to rise only after parenchymal injury [7]. Rise in serum creatinine is dependent on the degree of tubular necrosis [8]. Oxidative stress has also been reported in the tubular toxicity of gentamicin. Thus, gentamicin enhanced reactive oxygen species (ROS) formation [9, 10] and ROS-induced cell death was found to have a role in gentamicin-mediated acute renal failure [11, 12]. Therefore, treatment with antioxidants might be effective to ameliorate the damage [12, 13]. Several natural products have been used to protect from the toxicities induced by drugs. Astaxanthin is a red-pigment carotenoid occurring naturally in a wide variety of living organisms and classified as a xanthophylls [14]. It has a chemical structure similar to that of the familiar carotenoid β-carotene. It has been suggested that astaxanthin protects muscle cells from damaging effects [15]. The presence of hydroxyl (OH) and ketone (C=O) moieties on each ionone ring along with an extension of conjugated double bond system explained the potency of astaxanthin high antioxidant activity [16]. Astaxanthin has been reported to possess anti-inflammatory [17], hepatoprotective [18] and cardioprotective [19] activities. Though the antioxidant and hypolipidemic activity of astaxanthin was reported [17-19]. As an extension of our interested research program in therapeutic importance of astaxanthin [19]. We report herein, a facile

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route to evaluate the prophylactic effect of astaxanthin against gentamicin-induced nephrotoxicity in rats.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

- Astaxanthin (92%) was purchased from Sigma Chemical Co., St Louis, Missouri, USA. All other chemicals used were of analytical grade.
- Gentamicin sulphate vials was gifted from Alexandria Co. Egypt.

**Animals**

The experiments were carried out using male albino rats weighing 210±10 g purchased from Faculty of Veterinary Medicine, Cairo University. Animals were provided standard diet and water ad-libitum [20]. They were kept under constant environmental condition and observed daily throughout the experimental work. They were housed in polypropylene cages (47 cm x 34 cm x 20 cm) lined with husk, replaced every 24 h, under a 12:12 h light: dark cycle at around 22°C. All animal experiments were conducted according to the guidelines of Animal Care and Ethics Committee of the Faculty of Pharmacy, October 6 University, Egypt (registration No. 18229/2014).

**Treatments and Groups**

Nephrotoxicity was induced in rats by intraperitoneal injection of gentamicin in a dose of 80 mg/kg/day suspended in 1% tween 80 for 5 days [21].

Astaxanthin was brought into suspension in 1% tween 80 for intragastric intubation of rats.

Rats were divided into six groups, eight animals each. Selection of the doses of the used test substances was based on the published literature as follows:

- **Group I**: Normal control A (rats were given 3 ml of distilled water, orally)
- **Group II**: Normal control B (rats were given 3 ml of 1% tween 80, orally)
- **Group III**: Rats were treated with astaxanthin (50 mg/kg suspended in 1% tween 80, orally) for 15 days (19).
- **Group IV**: Rats were given gentamicin (80 mg/kg suspended in 1% tween 80, intraperitoneally) daily for the last 5 days of the experimental period [22].
- **Group V**: Rats were pretreated with astaxanthin (50 mg/kg suspended in 1% tween 80, orally) alone for 10 days then received both astaxanthin (50 mg/kg, orally) and gentamicin (80 mg/kg, intraperitoneally) for the other 5 days (prophylactic I).
- **Group VI**: Rats were simultaneously given astaxanthin (50 mg/kg, orally) and gentamicin (80 mg/kg, intraperitoneally) for 5 days followed by astaxanthin (50 mg/kg, orally) alone for other 10 days (prophylactic II).

On the 16th day of the experiment, blood samples were collected from the retro-orbital vein of each rat. Each sample was collected into 2 heparinized tubes. Blood in the first heparinized samples were divided into 2 aliquots; the first aliquot was used for determination of GPx and CAT activities. The second aliquot was hemolyzed using bidistilled water and the hemolysate of each sample was divided into two portions; the first portion was treated with chloroform/ethanol (3:5 V/V) mixture to precipitate and the resultant supernatant was used for the determination of SOD activity. The second portion was treated with 0.1M sodium phosphate buffer (pH 7.5) and the resultant supernatant was used for the determination of GR activity. Hemoglobin levels were determined in the heparinized blood samples and used in the calculation of the enzyme activity. The second part of heparinized blood samples were centrifuged at 1000xg for 20 min. The separated plasma was used for the estimation of sodium, potassium, creatinine, urea, TNF-α, NO, TBARs, TAS and HP as well as total protein.

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The kidneys were excised immediately and washed off from blood with ice-cold physiological saline. Then, they were blotted between filter papers to absorb moisture. The kidneys were divided into two parts. The first part (10% of total organ) was homogenized in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for assessment of renal GSH, Pr-SHs, MDA, HP, SOD, GPx, CAT, GR, G6PD and total protein. The second part was put into 10% formalin bottle and used for histopathological examination.

**Biochemical Assays**

Hemoglobin (Hb %) was estimated according to Drabkin [23]. Plasma sodium was estimated by colorimetric method of Maruna and Trinders [24]. Plasma potassium was estimated by the method of Maruna [25]. Urea was estimated by the method of Natelson [26]. Plasma creatinine was determined using alkaline picrate method [27]. Plasma and renal MDA, HP and GSH were estimated by the methods of Yoshioka et al. [28]; Jiang et al. [29] and Sedlak and Lindsay, [30], respectively. Plasma TAS was estimated by the method of Miller [31] and expressed in terms of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. SOD activity was assayed by the method of tetrzolium salt for detection of superoxide radicals generated by red formazan dye reduction produced [32]. GPx activity was measured indirectly by a coupled reaction with GR [33]. GR activity was assayed as described by Horn and Burns [34], with some modifications, by measuring the oxidation of NADPH at 340 nm. The Catalase assay kit utilizes the peroxidative function of CAT for determination of enzyme activity [35]. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The generated formaldehyde is assayed spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. The level of protein thiols (Pr-SHs) was measured according to the method of Tukahara et al. [36]. Finally, the protein content of renal tissue was measured by applying the method of Lowry et al. [37].

Quantitative estimation of plasma tumor necrosis factor–alpha (TNF-α) was carried out using enzyme linked immunosorbent assay (ELISA) according to Corti et al. [38].
Plasma nitrate concentration as a stable end product of nitric oxide was estimated using the Griess reaction after quantitative conversion of nitrate to nitrite by nitrate reductase according to the method of Moshage et al. [39].

Glucose-6-phosphate dehydrogenase (G6PD) was estimated using the method of Beutler [40], where an increase in the absorbance was measured as the reaction started by the addition of glucose-6-phosphate.

**Histological Assessment**

Rats were sacrificed and renal tissues from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counterstained with Hematoxylin and Eosin (H&E) for light microscopic analysis according to the method of Bancroft and Steven [41]. The slides were coded and examined by a histopathologist who was ignorant about the treated groups, after which photographs were taken.

**Statistical Analysis**

All data were expressed as mean ± SD. All analyses utilized SPSS 13.0 statistical package for Windows (SPSS, 13.0 software, Inc., Chicago, IL, 2009) [42]. A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value < 0.05 was accepted as statistically significant.

**RESULTS**

The changes in the levels of plasma urea and creatinine in untreated nephrotoxic rats were significantly (2-fold) increased when compared with normal rats. Treatment of nephrotoxic rats with astaxanthin (50 mg/kg) pre-and post-treatment significantly decreased the level of plasma urea (43.41 and 47.11%, respectively) and creatinine (43.00 and 55.95%, respectively) when compared with untreated nephrotoxic group.

Levels of plasma sodium and potassium are shown in Table 1. Plasma sodium and potassium levels in untreated nephrotoxic rats were significantly (1.59 and 1.48-folds, respectively) decreased when compared with normal rats. Treatment of nephrotoxic rats with astaxanthin (50 mg/kg) pre-and post-treatment significantly increased plasma sodium (49.39 and 32.53%, respectively) and potassium (36.69 and 22.98%, respectively) when compared with untreated nephrotoxic group (p<0.05). Gentamicin nephrotoxicity effect was controlled in the rats treated with astaxanthin as evidenced by restoring the levels of the renal biomarkers; urea, creatinine, sodium and potassium.

Rates of TNF-α, NO, MDA and TAS concentrations were used as a measure of inflammatory condition, lipid peroxidation and antioxidanistatus. Table 2 showed the changes in the levels of TNF-α, NO, MDA and TAS in all groups. The TNF-α, NO, MDA and TAS levels were similar in the control and astaxanthin (50 mg/kg) groups (p>0.05). The administration of astaxanthin (50 mg/kg) alone did not increase lipid peroxides when compared with the control group. Gentamicin induced a statistically significant increase in the formation of TNF-α, NO and MDA as well as significant decrease in TAS levels when compared with the control group (p <0.05). Pretreatment of rats with astaxanthin (50 mg/kg) significantly decreased TNF-α, NO and MDA as well as significant increase in TAS levels when compared with gentamicin treated group.

Levels of renal reduced glutathione (GSH) and protein thiols (Pr-SHs) are shown in Table 3. Renal GSH and Pr-SHs levels in untreated nephrotoxic rats were significantly decreased (1.78 and 1.61-folds, respectively) when compared with normal rats. Treatment of nephrotoxic rats with astaxanthin (50 mg/kg) pre-and post-treatment significantly increased renal GSH (76.70 and 70.45%, respectively) and Pr-SHs (69.80 and 53.95%, respectively) when compared with untreated nephrotoxic group.

Levels of renal MDA and HP in untreated nephrotoxic rats were significantly increased (1.90 and 1.64-folds, respectively) when compared with normal rats. Treatment of untreated nephrotoxic rats with astaxanthin (50 mg/kg) pre-and post-treatment significantly increased renal MDA (139.86 and 136.00 ± 4.97, respectively) and HP (87.27 ± 7.45, respectively) when compared with untreated nephrotoxic group.

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Sodium (mEq/L)</th>
<th>Potassium (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control A (distilled water-treated) 3 ml/kg</td>
<td>37.21 ± 3.12</td>
<td>0.78 ± 0.08</td>
<td>138.84 ± 5.40</td>
<td>7.32 ± 1.07</td>
</tr>
<tr>
<td>Normal control B (1% tween 80-treated) 3 ml/kg</td>
<td>36.66 ± 4.60</td>
<td>0.77 ± 0.07</td>
<td>136.00 ± 4.97</td>
<td>7.25 ± 0.79</td>
</tr>
<tr>
<td>Astaxanthin (50 mg/kg) suspended in 1% tween 80</td>
<td>33.15 ± 3.00</td>
<td>0.75 ± 0.10</td>
<td>139.86 ± 8.90</td>
<td>7.35 ± 0.58</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg) suspended in 1% tween 80</td>
<td>81.15 ± 5.68abc</td>
<td>1.93 ± 0.13abc</td>
<td>87.27 ± 7.45abc</td>
<td>4.96 ± 1.36abc</td>
</tr>
<tr>
<td>Astaxanthin + Gentamicin (Prophylactic I)</td>
<td>35.23 ± 1.48abc</td>
<td>0.83 ± 0.00abc</td>
<td>130.38 ± 5.08abc</td>
<td>6.78 ± 0.60abc</td>
</tr>
<tr>
<td>Gentamicin + Astaxanthin (Prophylactic II)</td>
<td>38.23 ± 4.00abc</td>
<td>1.08 ± 0.08abc</td>
<td>115.66 ± 9.25abc</td>
<td>6.10 ± 2.28abc</td>
</tr>
</tbody>
</table>

Gentamicin (80 mg/kg) was given intraperitoneally as a single daily dose for 5 days. Astaxanthin (50 mg/kg) was given orally as a single daily dose for 15 days. a: significant from normal control A group; b: significant from normal control B group; c: significant from astaxanthin (50 mg/kg) supplement group; d: significant from gentamicin (80 mg/kg) induced-renal toxicity group; e: significant from Prophylactic I group. Values are statistically significant at *P<0.05.

n= number of rats in each group.
Table 2. The effect of astaxanthin on plasma levels of TNF-α, NO, MDA and total antioxidant status (TAS) in gentamicin-induced nephrotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups (n= 8)</th>
<th>TNF-α (pg/ml)</th>
<th>NO (µmol/L)</th>
<th>MDA (nmole/L)</th>
<th>TAS (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control A (distilled water-treated) 3 ml/kg</td>
<td>34.75± 3.00</td>
<td>19.60 ± 1.75</td>
<td>17.55±1.64</td>
<td>29.45±2.55</td>
</tr>
<tr>
<td>Normal control B (1% tween 80-treated) 3 ml/kg</td>
<td>33.50 ± 2.36</td>
<td>19.00±1.44</td>
<td>18.90 ± 3.76</td>
<td>28.45±1.75</td>
</tr>
<tr>
<td>Astaxanthin (50 mg/kg) suspended in 1% Tween 80 1% tween 80</td>
<td>30.16± 2.50</td>
<td>17.70±2.07</td>
<td>16.23±1.22</td>
<td>29.00± 3.09</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg) suspended in 1% Tween 80</td>
<td>66.75± 4.87abc</td>
<td>53.50±5.11abc</td>
<td>41.48±3.56abc</td>
<td>17.64±1.85abc</td>
</tr>
<tr>
<td>Astaxanthin + Gentamicin (Prophylactic I)</td>
<td>35.29±3.62d</td>
<td>18.86±3.20d</td>
<td>21.86±2.00d</td>
<td>27.88±2.40d</td>
</tr>
<tr>
<td>Gentamicin + Astaxanthin (Prophylactic II)</td>
<td>39.17±2.33d</td>
<td>24.45±2.31de</td>
<td>28.20±1.73de</td>
<td>35.88±2.25de</td>
</tr>
</tbody>
</table>

Gentamicin (80 mg/kg) was given intraperitoneally as a single daily dose for 5 days. Astaxanthin (50 mg/kg) was given orally as a single daily dose for 15 days. a: significant from normal control A group; b: significant from normal control B group; c: significant from astaxanthin (50 mg/kg) supplement group; d: significant from gentamicin (80 mg/kg) induced-renal toxicity group; e: significant from Prophylactic I group. Values are statistically significant at *P<0.05.

n= number of rats in each group.

Table 3. The effect of astaxanthin on renal levels of reduced glutathione (GSH), protein thiols (Pr-SHs), malondialdehyde (MDA) and hydroperoxide (HP) in gentamicin-induced nephrotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups (n= 8)</th>
<th>GSH (µg/g protein)</th>
<th>Pr-SHs (µg/g protein)</th>
<th>MDA (mg/g tissue)</th>
<th>HP (mmole/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control A (distilled water-treated) 3 ml/kg</td>
<td>3.14 ± 0.31</td>
<td>9.20 ± 0.24</td>
<td>2.54±0.54</td>
<td>0.62 ± 0.02</td>
</tr>
<tr>
<td>Normal control B (1% Tween 80-treated) 3 ml/kg</td>
<td>3.18 ± 0.11</td>
<td>9.00±0.17</td>
<td>2.55 ± 0.22</td>
<td>0.64 ± 0.05</td>
</tr>
<tr>
<td>Astaxanthin (50 mg/kg) suspended in 1% Tween 80 1% Tween 80</td>
<td>3.22±0.25</td>
<td>9.17±0.35</td>
<td>2.51±0.17</td>
<td>0.64 ± 0.04</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg) suspended in 1% Tween 80</td>
<td>1.76±0.15abc</td>
<td>5.69±0.45abc</td>
<td>4.86±0.94abc</td>
<td>1.02 ± 0.09abc</td>
</tr>
<tr>
<td>Astaxanthin + Gentamicin (Prophylactic I)</td>
<td>3.11±0.33d</td>
<td>9.15±0.26d</td>
<td>2.50±0.44d</td>
<td>0.62 ± 0.06d</td>
</tr>
<tr>
<td>Gentamicin + Astaxanthin (Prophylactic II)</td>
<td>3.00±0.28d</td>
<td>8.76±0.37d</td>
<td>2.65±0.53d</td>
<td>0.69±0.04d</td>
</tr>
</tbody>
</table>

Gentamicin (80 mg/kg) was given intraperitoneally as a single daily dose for 5 days. Astaxanthin (50 mg/kg) was given orally as a single daily dose for 15 days. a: significant from normal control A group; b: significant from normal control B group; c: significant from astaxanthin (50 mg/kg) supplement group; d: significant from gentamicin (80 mg/kg) induced-renal toxicity group. Values are statistically significant at *P<0.05.

n= number of rats in each group.

nephrotic rats with astaxanthin (50 mg/kg) pre-and post-treatment was significantly decreased renal MAD (51.44 and 54.52%, respectively) and HP (60.78 and 67.64%, respectively) when compared with untreated nephrotic group (Table 3).

Tables 4 and 5 showed the changes in the levels of blood and renal enzymatic antioxidants; SOD, CAT, GPx and GR as well as renal G6PD in all groups. The SOD, CAT, GPx, GR and G6PD levels were similar in the control and astaxanthin (50 mg/kg) groups (p <0.05). Gentamicin induced a statistically significant decrease in blood and renal SOD, CAT, GPx and GR as well as renal G6PD when compared with the control group (p <0.05). Interestingly, blood and renal SOD, CAT, GPx and GR as well as renal G6PD levels increased significantly in rats treated with astaxanthin (50 mg/kg) plus gentamicin when compared with the gentamicin treated group. This shows that astaxanthin (50 mg/kg) pre-or post-treatment could inhibit gentamicin-induced decrease in blood and renal SOD, CAT, GPx and GR as well as renal G6PD.

The level of hemoglobin (Hb) and renal protein are shown in Tables 4 and 5. The level of hemoglobin (Hb) and renal protein in untreated nephrotic rats significantly (p<0.05) (1.13 and 1.29-folds, respectively) decreased when compared with normal rats. Treatment of nephrotic rats with astaxanthin (50 mg/kg) pre-and post-treatment significantly increased hemoglobin (Hb) (10.25 and 8.56%, respectively) and renal protein (37.41 and 15.19%, respectively) when compared with untreated nephrotic group.

Histopathological Examination of Kidney Tissues

Figs. (1-A, B and C) show the histological section in the kidney of normal controls A and B as well as astaxanthin treated group. According to microscopic examinations no pathological lesions were elicited in the kidney of normal controls A and B. Also, the histoarchitectural patterns of kidneys were almost normal in rats treated with astaxanthin alone groups (1-3). Figures (2-D, E and F) show the histological section in the kidney of experimental groups (4-6).
Table 4. The effect of astaxanthin on blood superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and hemoglobin in gentamicin-induced nephrotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>SOD (U/g. Hb)</th>
<th>GPx (U/g. Hb)</th>
<th>CAT (U/g. Hb)</th>
<th>Hb%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control A (distilled water-treated) 3 ml/kg</td>
<td>15.25 ± 2.08</td>
<td>132.05 ± 8.13</td>
<td>21.64±2.18</td>
<td>12.75 ± 2.25</td>
</tr>
<tr>
<td>Normal control B (1% tween 80-treated) 3 ml/kg</td>
<td>14.70 ± 1.65</td>
<td>128.96±8.69</td>
<td>19.53± 3.06</td>
<td>12.54 ± 1.48</td>
</tr>
<tr>
<td>Astaxanthin (50 mg/kg) suspended in 1% tween 80</td>
<td>15.00 ± 3.07</td>
<td>130.20±11.21</td>
<td>24.60±2.09</td>
<td>12.64± 1.79</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg) suspended in 1% tween 80</td>
<td>7.53± 0.94</td>
<td>85.71 ± 6.15</td>
<td>11.84±2.06</td>
<td>11.21 ± 2.33</td>
</tr>
<tr>
<td>Astaxanthin + Gentamicin (Prophylactic I)</td>
<td>14.66±1.26</td>
<td>118.09±9.26</td>
<td>24.45±4.19</td>
<td>12.36±2.40</td>
</tr>
<tr>
<td>Gentamicin + Astaxanthin (Prophylactic II)</td>
<td>14.51±2.10</td>
<td>109.94±10.07</td>
<td>18.36±2.08</td>
<td>12.17±1.96</td>
</tr>
</tbody>
</table>

Gentamicin (80 mg/kg) was given intraperitoneally as a single daily dose for 5 days. Astaxanthin (50 mg/kg) was given orally as a single daily dose for 15 days. a: significant from normal control A group; b: significant from normal control B group; c: significant from astaxanthin (50 mg/kg) supplement group; d: significant from gentamicin (80 mg/kg) induced-renal toxicity group; e: significant from Prophylactic I group. Values are statistically significant at *P<0.05. n= number of rats in each group.

Table 5. Renal levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD) and protein of control, astaxanthin and gentamicin treated rats.

<table>
<thead>
<tr>
<th>Groups (n=8)</th>
<th>SOD</th>
<th>GPx</th>
<th>CAT</th>
<th>GR</th>
<th>G6PD</th>
<th>Total protein mg/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control A (distilled water-treated) 3 ml/kg</td>
<td>11.27±1.54</td>
<td>6.74±0.87</td>
<td>44.37±3.25</td>
<td>0.42±0.02</td>
<td>1.84±0.87</td>
<td>82.14±3.54</td>
</tr>
<tr>
<td>Normal control B (1% tween 80-treated) 3 ml/kg</td>
<td>10.97±2.11</td>
<td>6.76±1.15</td>
<td>45.10±3.66</td>
<td>0.43±0.06</td>
<td>1.78±1.15</td>
<td>80.07±4.00</td>
</tr>
<tr>
<td>Astaxanthin (50 mg/kg) suspended in 1% tween 80</td>
<td>11.20±1.68</td>
<td>6.80±0.79</td>
<td>44.75±3.73</td>
<td>0.42±0.07</td>
<td>1.83±0.79</td>
<td>86.62±4.05</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg) suspended in 1% tween 80</td>
<td>6.35±1.44abc</td>
<td>3.11±0.65abc</td>
<td>27.16±2.40abc</td>
<td>0.21±0.08abc</td>
<td>0.94±0.65abc</td>
<td>63.75±5.11abc</td>
</tr>
<tr>
<td>Astaxanthin + Gentamicin (Prophylactic I)</td>
<td>11.10±2.07abc</td>
<td>6.58±1.20abc</td>
<td>42.80±4.00abc</td>
<td>0.38±0.06abc</td>
<td>1.70±1.20abc</td>
<td>87.60±4.83abc</td>
</tr>
<tr>
<td>Gentamicin + Astaxanthin (Prophylactic II)</td>
<td>10.84±1.25abc</td>
<td>6.44±0.96abc</td>
<td>38.25±2.48abc</td>
<td>0.30±0.04abc</td>
<td>1.65±0.96abc</td>
<td>73.58±3.96abc</td>
</tr>
</tbody>
</table>

Gentamicin (80 mg/kg) was given intraperitoneally as a single daily dose for 5 days. Astaxanthin (50 mg/kg) was given orally as a single daily dose for 15 days. a: significant from normal control A group; b: significant from normal control B group; c: significant from astaxanthin (50 mg/kg) supplement group; d: significant from gentamicin (80 mg/kg) induced-renal toxicity group. Values are statistically significant at *P<0.05. SOD: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min/mg protein; GPx: µg of GSH consumed/min mg protein; CAT: µmol of H2O2 utilized/min mg protein. The activities of GR were expressed as nmole of NADPH oxidized/min/mg protein and G6PD were expressed as nmole of NADPH formed/min/mg protein.

n= number of rats in each group.

Fig. (1 A, B and C): Sections in the kidneys of groups (1-3) show normal appearance; the glomeruli are normal (G), the tubules (T) are not dilated, no vacuolization of the lining cells at 400× magnification.
Kidney sections of the gentamicin-treated group show remarkable changes in the renal cortex versus control A and B groups of rats. These changes include: shrinkage of the glomeruli, tubular epithelial cells degeneration and tubular swelling (Fig. 2D). Administration of astaxanthin pre- or post-treatment to gentamicin-treated rats resulted in the improvement in the structure of proximal tubular cells (Figs. 2E and F). The histological examination of renal medulla revealed that the tubular cells and interstitial tissue in all treated groups had the normal appearance. The damage of tubular cells was not observed.

**DISCUSSION**

Amino-glycosides are widely applied antibiotics in medical treatment and are responsible for nephrotoxicity in 25-30% of treated cases [43]. Disturbance of glomerular filtration and changes in the proximal tubular structure are the key effects of gentamicin toxicity in some authors’ opinion [44, 45]. Gentamicin has been shown to cause damage when administered at doses 5-10 times the normal therapeutic dose [46]. Results of this study corroborated the previous reports in which gentamicin at dose of 80 mg/kg significantly produced nephrotoxicity [6, 47]. Studies showed that primary retention of gentamicin in proximal tubular cells following production of oxygen-associated metabolites and free radicals precede gentamicin-induced nephrotoxicity [48, 49].

Antioxidants are the chemical constituents that are used for inhibiting the tissue damage by countering the free radicals; most of the antioxidants available in the markets are from natural origin e.g. Vit-E, Vit-C, tocopherol, quercetin, β-carotene etc. [50-52]. In addition, there are reports which indicate that polyphenolic compounds like flavonoids and tannins are useful as antioxidants and organ protectants [53]. Astaxanthin (3,3’-dihydroxy-β-β-carotene-4,4’-dione) is a carotenoid, commonly found in marine environments. The presence of the hydroxyl and keto endings on each ionone ring (Fig. 3), explains some unique features of its structure, such as its ability to be esterified, its high anti-oxidant activity [50, 54] and a more polar configuration than other carotenoids [54]. Free astaxanthin is extremely sensitive to oxidation, however its esterified form, as found in nature in the *Haematococcus pluvialis* (*H. pluvialis*) microalg [55], is stable and displays higher bioavailability and potency [56].

In the present study, rats exposed to gentamicin (80 mg/kg) showed a significant reduction in plasma sodium and potassium levels and elevation in urea and creatinine levels. Kidney toxicity is associated with an increase in tissue lipid peroxidation as MDA, HP, TNF-α, NO and TAS levels were elevated causing oxidative stress. Oxidative stress of gentamicin appears also through reduction of plasma and renal GSH, Pr-SHs, SOD and CAT as well as renal GR and G6PD.

Gentamicin administration was reported to increase plasma urea and creatinine levels [6, 58] which is accompanied with a decrease in plasma sodium and potassium levels [58].

Gentamicin induced nephrotoxicity, glomerular changes and secondary tubular casts were evident by a significant increase in plasma urea and creatinine clearance [59], whilst
pre- or post-treatment of rats with astaxanthin (50 mg/kg) normalized plasma urea and creatinine levels. Astaxanthin has been reported to span the cell membrane bilayer (fat/water) because of its unique structure with polar terminal rings [60].

Reduction of plasma sodium and potassium levels in gentamicin treated group (80 mg/kg) is probably due to inactivation of Na/K ATPase during interaction of gentamicin with proximal tubular cells [59]. Also, simultaneous inhibition of different membrane protein species is not necessarily a prerequisite for the initial depression of Na/K ATPase and afterwards, multifactorial cell death processes [6].

The results showed that astaxanthin (50 mg/kg) could inhibit the reduction in plasma sodium and potassium levels in gentamicin-treated group. Oxidative stress of gentamicin resulted in production of free radicals which are involved in the regulation of cell proliferation and nephrotoxicity as well as reduction of sodium and potassium levels [6].

In the present study, oxidative stress caused by intraperitoneal administration of gentamicin was prevented by pre- or post-treatment of rats with the antioxidant astaxanthin (50 mg/kg). Many studies also showed that the administration of astaxanthin produces antioxidant activity under different conditions [59, 60]. The present study showed no significant change in plasma sodium and potassium concentration in astaxanthin pre- or post-treatment of rats when compared with negative control groups.

The results also showed that astaxanthin (50 mg/kg) could inhibit the plasma TNF-α and NO levels in gentamicin- treated group. Evidence indicates that free radicals, oxidative stress, and lipid peroxidation are present in renal damage [58]. Gentamicin causes changes in the equilibrium between antioxidant and prooxidant activity [6], favoring the latter, since it increased production of renal MDA and HP as well as reduced TAS, GSH, Pr-SHs, SOD, CAT, GR, and G6PD. It has been shown that pre- or post-treatment of rats with the antioxidant astaxanthin resulted in reduction of plasma MDA, HP, TNF-α and NO as well as elevation of plasma TAS and renal GSH, Pr-SHs, SOD, CAT, GR and G6PD. Tumor necrosis factor alpha (TNF-α), TGF-β1, and interleukin-6 (IL-6) are the most extensively studied mitogenic and fibrogenic factors. In addition to inhibiting their production, Hussein et al., [59] reported that, astaxanthin is also able to inhibit pro-inflammatory cytokine expression. Taken together, these results indicated that the anti-fibrotic effect of astaxanthin is associated with the blockade of mitogenic and/or fibrogenic signaling. TNF-α was reported to induce NO formation [18]. The increased NO production is recognized as an important mediator of physiological and pathological processes [61], as a result of inflammatory and destructive processes. In addition, astaxanthin is a potent reactive oxygen species (ROS) scavenger (17 and 18) and normalized the oxidative stress biomarkers (TAS, GSH, Pr-SHs, SOD, CAT, GR, G6PD, HP, NO, and MDA), resulting in reduced oxidative stress, which contributes to suppression of renal tissues inflammation by gentamicin administration. In the present study, the significant decrease in TAS, GSH, Pr-SHs, SOD, CAT, GR, G6PD activities was detected after gentamicin-administration.

The reduction in TAS, GSH, Pr-SHs, SOD, CAT, GR, G6PD activities in gentamicin-induced renal toxicity in rats when compared with normal rats was reported in this study due to production of NO as a result of inflammation and destructive processes [62]. The oxidative stress on the renal cells was increased leading to depletion of antioxidant enzymes that scavenge the toxic superoxide and hydrogen peroxide radicals. On the other hand, astaxanthin-treated groups showed a significant increase in the TAS, GSH, Pr-SHs, SOD, CAT, GR, G6PD activities of the gentamicin-induced nephrotoxicity rats. It was reported that the in vivo antioxidant property of astaxanthin probably arises from its ability to protect against the molecular effects of lipid peroxidation, free radicals and ROS, and it also delays the progress of many chronic diseases [63, 64].

Gentamicin-intoxicated rats showed a severe decrease in the activities of renal G6PD and GR. G6PD is an important enzyme in pentose phosphate pathway, which generates NADPH from NADP+. NADPH reducing equivalents are necessary to keep GSH in its reduced form through the enzyme GR. Glutathione reductase catalyzes the reduction of GSSG to GSH. NADPH is also important for the activity of catalase [65]. The decreased activity of G6PD in gentamicin-treated rats could be due to nephrotoxicity and formation of free radicals that maintains the tertiary structure of G6PD [66], which in-turn reduces the generation of NADPH and may consequently decrease the activity of GR, the enzyme that catalyses the regeneration of GSH from GSSG [67]. Pre- or post-treatment of rats with astaxanthin (50 mg/kg) in gentamicin intoxicated rats significantly increased the activities of these glutathione metabolizing enzymes in kidney tissue via their co-ordinating antioxidant activity.

Histological studies confirmed renal protective effect of astaxanthin. Kidney sections of gentamicin-treated rats showed inflammation, cell infiltration and necrosis. Pre- or post-treatment of rats with astaxanthin (50 mg/kg) almost normalized these effects in the histarchitecture of the kidney. Furthermore, the severe fatty degenerative changes and vacuolization in the kidney of rats caused by gentamicin were improved in the gentamicin- treated groups. Therefore, from this study pre- or post-treatment of rats with astaxanthin (50 mg/kg) could be a renal protective against gentamicin-induced nephrotoxicity in rats. Many authors reported the degeneration and desquamation of epithelial cells of proximal tubules caused by gentamicin [66, 67]. This is in concordance with the results reported in this study.

In addition, the most novel and relevant finding was that astaxanthin supplementation was accompanied by the alleviation of oxidative stress produced by gentamicin intoxication in this model. Astaxanthin was also able to reduce newly inflammatory cells, which was in agreement with the results of renal functional markers and the kidney lipid peroxidation and antioxidant status. Finally, the antioxidant and renal protective effects of astaxanthin might be associated with its structure-antioxidant relationship.

In conclusion, the present study showed that astaxanthin has a powerful renal protective activity against gentamicin induced nephrotoxicity. These effects could be due to membrane protective action of astaxanthin by scavenging the free radicals and its antioxidant action.
LIST OF ABBREVIATIONS

TNF-α = Tumor necrosis factor alpha
MDA = Malondialdehyde
TGF-β1 = Transforming growth factor- β1
HP = Hydroperoxide
TAS = Total antioxidant status
GSH = Reduced glutathione
Pr-SHs = Protein thiols
G6PD = Glucose-6-phosphate dehydrogenase
SOD = Superoxide dismutase
GPx = Glutathione peroxidase
CAT = Catalase
GR = Glutathione reductase
ROS = Reactive oxygen species

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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REFERENCES

[17] Ohtaga, K.; Shiratori, K. Effects of astaxanthin on lipopolysaccha-
[18] Li, W.; Hlisten, A. α-tocopherol and astaxanthin decrease macro-
phage infiltration, apoptosis and vulnerability in atheroma of hyper-
[19] Hussein, M.A. Cardioprotective effects of astaxanthin against iso-
tional Research Council.
[21] Said, M.M. The protective effect of eugenol against gentami-
[31] Miller, N.; Rice-Evans, C.; Davies, M.; Gopjathnan, V.; Milner, A. A novel method for measuring antioxidant capacity and its applica-
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