PROTECTIVE EFFECT OF DIETARY FISH OIL ON CYCLOSPORINE A-INDUCED NEPHROTOXICITY IN RATS

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A B S T R A C T

Cyclosporine A (CsA) is a lipophilic cyclic polypeptide composed of 11 amino acids, seven of which are N-methylated. It has been utilized clinically as a potent immunosuppressant to prevent allograft rejection in various organ transplantations and to treat systemic and local autoimmune disorders, but it can impair renal function. CsA-induced nephrotoxicity results from increased production of free radical species in the kidney. The present study was designed to investigate the possible protective effect of dietary fish oil (FO) on CsA-induced nephrotoxicity in rats. Eighty male rats were divided into four equal groups. Group 1 rats received no drugs and served as control, group 2 normal rats were treated with (dietary fish oil) omega-3 fatty acids 270 mg/kg b.w oral dose daily, group 3 rats treated with CsA (25 mg/kg body weight, orally for 21 days) to induce nephrotoxicity, groups 4 rats received dietary fish oil for 21 days before, 21 days concurrently during CsA administration and 21 days later after nephrotoxicity induction. Blood samples for serum separation and kidney tissue specimens were collected three times at weekly interval from the last dose of CsA administration. Serum glucose, total cholesterol, triacylglycerols, phospholipids, creatinine, uric acid, urea, sodium, potassium, inorganic phosphorus, total protein, albumin, haptoglobin levels, lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) activities were also determined. Moreover, kidney tissue malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAO) levels, antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were also determined. The results revealed that CsA-induced nephrotoxicity caused a significant increase in serum glucose, renal functions tests, lipid profiles and serum marker enzymes (LDH and GGT) with a significant decrease in serum total protein, albumin and electrolytes concentrations, which were reversed upon treatment with dietary fish oil. In addition, CsA administration induced a significant elevation in lipid peroxidation (MDA) along with a significant decrease in antioxidant enzyme activities, non enzymatic antioxidant, total antioxidant capacity and nitric oxide level in the rat kidney. Meanwhile, dietary fish oil administration improved renal functions, by a significant decrease in peroxidative levels and increase in antioxidant status. These results indicate the renoprotective potential and usefulness of dietary fish oil, as an excellent source of antioxidants, in modulating CsA-induced nephrotoxicity.

Keywords: Cyclosporine A; antioxidant enzymes, lipid peroxidation; Nephrotoxicity; Dietary fish oil.

1. INTRODUCTION

Cyclosporine (Cs), a cyclic decapeptide obtained from extracts of soil fungus tolypocladium inflatum gams, is the most effective and widely used first-line immunosuppressant in solid organ transplantation and autoimmune diseases [1]. Nephrotoxicity is the main effects of cyclosporine A (CsA) treatment. Although the mechanisms of nephrotoxicity are not completely defined, there is an evidence that suggests the role of reactive oxygen species (ROS) in its pathogenesis. It has been demonstrated in numerous in vivo and
in vitro experiments that CsA induced renal failure and increased the synthesis of ROS, thromboxane (TX) and lipid peroxidation products in the kidney. Furthermore, CsA modified the expression and activity of several renal enzymes (ciclooxygenase, superoxidedismutase, catalase and glutathione-peroxidase) [2]. It is reported that the level of free radicals in urine was increased significantly following CsA treatment. ROS could also be derived either directly from CsA or during its metabolism by the cytochrome P450 system [3]. Acute CsA treatment induces reversible reduction of the glomerular filtration rate (GFR) and renal blood flow that is related to afferent arteriolar vasoconstriction. This may be referred to an increase in vasoconstrictors factors such as endothelin, thromboxane, angiotensin II and/or a decrease in vasodilators factors such as prostacyclin and nitric oxide (NO) [4]. In addition, CsA has been reported to block mitochondrial calcium release inducing an increase in intracellular free calcium that could account for the CsA vasoconstriction effect. In past few years much interest has been centered on the role of naturally occurring dietary substances for the control and management of various chronic diseases. A number of investigations have demonstrated that diet supplemented with fish oil (FO) enriched in O-3 fatty acids has profound beneficial health effects against various pathologies [5] including cardiovascular diseases, respiratory diseases, diabetes, depression, cancers, inflammatory and immune renal disorders [6]. Reports showed that FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity [7]. Fish oil is rich sources of the essential fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Compared to saturated fats, poly unsaturated fatty acids (PUFAs) are more readily used for energy when initially ingested. Increasing the degree of unsaturation at a given carbon chain length increases the relative mobility of stored fat, making PUFAs more bioavailable [8]. Previous studies also showed that fish oil inhibited growth of breast cancer cells and hepatocarcinoma in rats [9]. Moreover, fish oil as n-3 polyunsaturated fatty acid, eicosapentaenoic and docosahexaenoic acid inhibited human lung carcinoma cell growth and prostate cancer [10]. FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity [6]. The most common dose-limiting effects of cyclosporin are hypertension and impaired renal functions. These effects appear to be in part thromboxane mediated [11]. Fish oil inhibits TXA2 production and reduces both the hypertensive and nephrotoxic effects of cyclosporine [12]. An additional and important factor in the nephroprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, fish oil could be a factor contributing to its nephroprotective ability through inhibition of cytochrome P-450 aromatase [13].

2. MATERIALS AND METHODS

2.1. Experimental animals:

Eighty white male albino rats of 12-16 weeks old and weighting 220 - 250 gm were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad- libitum. The animals were left 14 days for acclimatization before the beginning of the experiment.

2.2. Drug and antioxidants:

The drug and antioxidant compounds used in the present study were: 1. Cyclosporine (CsA): Cyclosporine (CsA) presents in the form of soft gelatine capsules containing 50 mg cyclosporine under traditional name (Sandimmune®, Neoral®) was obtained from (Novartis Pharma AG, Basilea, Suiza) and freshly dissolved in propylene glycol.
Protective effect of dietary fish oil on cyclosporine a-induced nephrotoxicity in rats

Nephrotoxicity was induced in rats after oral administration of cyclosporine (CsA) at a dose of 25 mg/kg body weight/day for 21 days. 2. Omega-3 fatty acids: Omega-3 fatty acids was chosen to be within the therapeutic range levels reported in the pamphlet, omega-3 fatty acids manufactured by South Egypt Drug Industries Co. (SEDICO), 6 October City-Egypt. The concentration of omega-3 fatty acids 1000mg and present in the soft gelatine capsulated form. Omega-3 fatty acids was dissolved in propylene glycol and administered in oral daily doses using stomach tube. Doses of omega-3 fatty acids was administered orally in a daily dose of 270 mg/kg body weight.

2.3. Experimental design:
After acclimatization to the laboratory conditions, the animals were randomly divided into four groups (twenty rats each) as follows: Group I: Rats received no drugs served as control for all experimental groups. Group II: Rats administered omega-3 fatty acids 270 mg/kg b.w oral dose daily all over the experimental periods (9 weeks). Group III: Rats were administered cyclosporine A (25 mg/kg body weight) start from the day 22 of experiment, once daily by oral gavage, for a period of 21 days. Group IV: Rats received oral administration of Omega-3 fatty acids 270 mg/kg b.w oral dose daily for 21 days before cyclosporine A, then for 21 days concomitant with cyclosporine A administration as in group III and the administration of cyclosporine A continued for 21 days later (end of experiment, 9 weeks).

2.4. Sampling:
Blood samples and renal tissue specimens were collected from all animals groups, three times during the experiment at 1st, 2nd and 3rd weeks from the last dose of CsA administration.

2.4.1. Blood samples:
Blood samples were collected by ocular vein puncture in dry, clean and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes. The clear serum was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

2.4.2. Renal tissue specimens:
Rats killed by decapitation. The kidney specimen quickly removed, cleaned by rinsing with cold saline and stored at -20°C. Briefly, renal tissues was minced into small pieces, homogenized with ice cold 0.05 M potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were be centrifuged at 6000 r.p.m for 15 minutes at 4°C until used for subsequent biochemical analysis.

2.5. Biochemical analysis:
Serum glucose, total protein, albumin, total cholesterol, triacylglycerols, phospholipids, urea, uric acid, creatinine, sodium, potassium, inorganic phosphorus and haptoglobin concentrations, lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) activities were determined according to the methods described by [14-27] respectively. Moreover, the supernatant of renal tissue homogenate were used for the determination of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAO) and antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) according to the methods described by [28-34] respectively.

2.6. Statistical analysis:
The results were expressed as mean±SE and statistical significance was evaluated by one way ANOVA using SPSS (version 10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $p < 0.05$. 

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Table (1): Effect of fish oil administration on serum glucose, lipid profiles and renal function tests in normal and cyclosporine-induced nephrotoxicity in rats:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glucose (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipids (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
<td></td>
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<tr>
<td><strong>1st Week</strong></td>
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</tr>
<tr>
<td>C</td>
<td>72.00±2.27 b</td>
<td>97.23±9.71 b</td>
<td>114.18±4.99 b</td>
<td>134.39±13.19 b</td>
<td>0.86±0.02 b</td>
<td>2.51±0.13 b</td>
<td>28.71±2.99 c</td>
</tr>
<tr>
<td>FO</td>
<td>79.25±4.71 c</td>
<td>87.90±3.88 b</td>
<td>73.67±9.42 c</td>
<td>127.23±7.39 b</td>
<td>0.78±0.03 bc</td>
<td>2.61±0.17 b</td>
<td>29.46±2.77 c</td>
</tr>
<tr>
<td>CsA</td>
<td>147.33±15.00 a</td>
<td>159.28±13.46 a</td>
<td>145.82±11.91 a</td>
<td>195.07±8.35 a</td>
<td>1.86±0.05 b</td>
<td>4.86±0.74 a</td>
<td>60.32±3.52 a</td>
</tr>
<tr>
<td>CsA+FO</td>
<td>104.25±1.89 b</td>
<td>94.07±6.22 b</td>
<td>68.73±2.72 c</td>
<td>127.23±4.43 b</td>
<td>0.84±0.02 b</td>
<td>2.66±0.18 b</td>
<td>29.68±2.68 c</td>
</tr>
<tr>
<td><strong>2nd Week</strong></td>
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</tr>
<tr>
<td>C</td>
<td>95.00±3.81 d</td>
<td>115.53±5.64 b</td>
<td>74.56±5.56 b</td>
<td>135.19±16.53 b</td>
<td>0.75±0.01 c</td>
<td>1.89±0.14 b</td>
<td>27.97±2.61 b</td>
</tr>
<tr>
<td>FO</td>
<td>109.75±5.85 c</td>
<td>111.16±4.05 b</td>
<td>90.51±4.49 b</td>
<td>119.91±16.11 b</td>
<td>0.87±0.05 b</td>
<td>2.07±0.06 b</td>
<td>13.67±1.16 c</td>
</tr>
<tr>
<td>CsA</td>
<td>187.50±2.60 a</td>
<td>259.32±17.04 a</td>
<td>137.98±9.79 a</td>
<td>232.81±2.76 a</td>
<td>1.37±0.04 a</td>
<td>4.30±0.72 a</td>
<td>48.88±0.28 a</td>
</tr>
<tr>
<td>CsA+FO</td>
<td>121.00±2.38 b</td>
<td>66.84±6.05 d</td>
<td>83.55±12.33 b</td>
<td>137.26±4.07 b</td>
<td>0.87±0.02 b</td>
<td>2.23±0.13 b</td>
<td>16.49±1.18 c</td>
</tr>
<tr>
<td><strong>3rd Week</strong></td>
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<tr>
<td>C</td>
<td>104.75±4.59 b</td>
<td>97.26±2.39 b</td>
<td>61.65±6.80 b</td>
<td>139.33±18.46 b</td>
<td>0.65±0.02 bc</td>
<td>1.84±0.14 b</td>
<td>26.38±1.46 b</td>
</tr>
<tr>
<td>FO</td>
<td>121.00±2.48 b</td>
<td>89.97±9.45 b</td>
<td>59.24±7.06 b</td>
<td>131.21±10.08 b</td>
<td>0.84±0.05 bc</td>
<td>1.60±0.07 b</td>
<td>20.45±1.83 bc</td>
</tr>
<tr>
<td>CsA</td>
<td>133.50±26.47 a</td>
<td>163.74±25.60 a</td>
<td>207.97±19.69 a</td>
<td>207.64±20.99 a</td>
<td>1.72±0.27 a</td>
<td>4.92±0.97 a</td>
<td>37.86±1.07 a</td>
</tr>
<tr>
<td>CsA+FO</td>
<td>120.75±5.22 a</td>
<td>78.29±3.87 b</td>
<td>67.60±8.30 b</td>
<td>131.21±9.39 b</td>
<td>1.01±0.08 b</td>
<td>1.89±0.17 b</td>
<td>20.79±1.51 bc</td>
</tr>
</tbody>
</table>

(C: Control Normal group, FO: Fish oil group, CsA: Cyclosporine A group, CsA+FO: Cyclosporine A + Fish oil group). Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<$0.05).
### Protective effect of dietary fish oil on cyclosporine a- induced nephrotoxicity in rats

Table (2): Effect of fish oil administration on serum electrolytes, proteins and Haptoglobin concentrations, LDH and GGT activities in normal and cyclosporine- induced nephrotoxicity in rats:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium (meq/L)</th>
<th>Potassium (meq/L)</th>
<th>Inorganic phosphorus (mg/dl)</th>
<th>Total protein (mg/dl)</th>
<th>Albumin (mg/dl)</th>
<th>Haptoglobin (mg/dl)</th>
<th>LDH (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
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<td><strong>1st Week</strong></td>
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</tr>
<tr>
<td>C</td>
<td>142.30 ± 2.02</td>
<td>6.70 ± 0.13</td>
<td>7.80 ± 0.34</td>
<td>6.23 ± 0.20</td>
<td>3.62 ± 0.23</td>
<td>70.70 ± 15.01</td>
<td>1191.72 ± 196.91</td>
<td>17.33 ± 0.43</td>
</tr>
<tr>
<td>FO</td>
<td>143.78 ± 1.10</td>
<td>6.72 ± 0.10</td>
<td>2.53 ± 0.74</td>
<td>6.38 ± 0.10</td>
<td>3.82 ± 0.15</td>
<td>66.97 ± 24.14</td>
<td>781.17 ± 246.43</td>
<td>15.29 ± 0.71</td>
</tr>
<tr>
<td>CsA</td>
<td>130.55 ± 3.35</td>
<td>3.95 ± 0.40</td>
<td>1.57 ± 0.17</td>
<td>4.07 ± 0.09</td>
<td>3.17 ± 0.11</td>
<td>173.17 ± 4.19</td>
<td>2475.04 ± 85.37</td>
<td>66.89 ± 3.80</td>
</tr>
<tr>
<td>CsA+FO</td>
<td>139.43 ± 0.81</td>
<td>6.09 ± 0.50</td>
<td>1.40 ± 0.07</td>
<td>6.43 ± 0.15</td>
<td>3.75 ± 0.08</td>
<td>88.97 ± 15.70</td>
<td>1321.15 ± 129.28</td>
<td>12.57 ± 0.74</td>
</tr>
<tr>
<td><strong>2nd Week</strong></td>
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<tr>
<td>C</td>
<td>145.40 ± 0.43</td>
<td>6.64 ± 0.23</td>
<td>2.82 ± 0.15</td>
<td>6.24 ± 0.22</td>
<td>3.69 ± 0.10</td>
<td>55.25 ± 3.32</td>
<td>1542.10 ± 113.74</td>
<td>12.88 ± 1.29</td>
</tr>
<tr>
<td>FO</td>
<td>143.43 ± 1.10</td>
<td>6.33 ± 0.32</td>
<td>2.89 ± 0.51</td>
<td>6.28 ± 0.18</td>
<td>3.70 ± 0.19</td>
<td>26.07 ± 1.22</td>
<td>1584.0 ± 117.65</td>
<td>14.96 ± 0.76</td>
</tr>
<tr>
<td>CsA</td>
<td>131.50 ± 0.87</td>
<td>5.87 ± 0.44</td>
<td>3.12 ± 0.03</td>
<td>79.63 ± 27.04</td>
<td>47.08 ± 15.93</td>
<td>594.98 ± 81.65</td>
<td>2537.79 ± 21.03</td>
<td>69.16 ± 1.10</td>
</tr>
<tr>
<td>CsA+FO</td>
<td>140.05 ± 0.46</td>
<td>1.89 ± 0.17</td>
<td>3.20 ± 0.03</td>
<td>48.78 ± 15.93</td>
<td>594.98 ± 81.65</td>
<td>16.85 ± 0.36</td>
<td>44.70 ± 20.35</td>
<td>14.46 ± 0.71</td>
</tr>
<tr>
<td><strong>3rd Week</strong></td>
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</tr>
<tr>
<td>C</td>
<td>146.05 ± 0.72</td>
<td>6.00 ± 0.14</td>
<td>4.64 ± 0.26</td>
<td>5.61 ± 0.06</td>
<td>3.61 ± 0.21</td>
<td>24.87 ± 0.82</td>
<td>1629.12 ± 85.69</td>
<td>15.36 ± 0.33</td>
</tr>
<tr>
<td>FO</td>
<td>142.68 ± 1.40</td>
<td>3.12 ± 0.52</td>
<td>4.02 ± 0.36</td>
<td>64.25 ± 12.64</td>
<td>2329.34 ± 47.93</td>
<td>60.51 ± 4.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsA</td>
<td>132.15 ± 3.45</td>
<td>1.99 ± 0.46</td>
<td>3.03 ± 0.03</td>
<td>64.25 ± 12.64</td>
<td>716.41 ± 17.95</td>
<td>14.46 ± 0.71</td>
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</tr>
<tr>
<td>CsA+FO</td>
<td>143.75 ± 0.99</td>
<td>1.61 ± 0.37</td>
<td>3.11 ± 0.06</td>
<td>44.70 ± 20.35</td>
<td>716.41 ± 17.95</td>
<td>14.46 ± 0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(C: Control Normal group, FO: fish oil group, CsA: Cyclosporine A group, CsA+FO: Cyclosporine A + fish oil group). Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at ($P<0.05$).
Table (3): Effect of fish oil administration on renal tissue L-MDA, CAT, SOD, GPx, GSH, NO and TAOC in normal and cyclosporine-induced nephrotoxicity in rats:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>(L-MDA) (nmol/g.tissue)</th>
<th>CAT (U/g. tissue)</th>
<th>(SOD) (U/g. tissue)</th>
<th>GPx (GSH Consumed/min/mg protein)</th>
<th>(GSH) (mg/g.tissue)</th>
<th>(NO) (µmol/g.tissue)</th>
<th>(TAO) (mmol/g.tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>31.23±8.47 c</td>
<td>2.10±0.21 a</td>
<td>36.36±3.22 a</td>
<td>0.46±0.002 a</td>
<td>64.33±2.33 a</td>
<td>115.74±1.62 a</td>
<td>1.11±0.04 a</td>
</tr>
<tr>
<td></td>
<td>1st Week</td>
<td>24.73±3.21 c</td>
<td>2.38±0.03 a</td>
<td>21.55±1.41 c</td>
<td>0.45±0.013 a</td>
<td>35.00±2.08 b</td>
<td>106.48±3.98 ab</td>
<td>0.64±0.03 c</td>
</tr>
<tr>
<td></td>
<td>FO</td>
<td>106.27±9.21 a</td>
<td>1.73±0.16 a</td>
<td>24.38±4.12 bc</td>
<td>0.42±0.009 b</td>
<td>29.67±3.38 b</td>
<td>66.66±6.41 b</td>
<td>0.71±0.02 bc</td>
</tr>
<tr>
<td></td>
<td>CsA</td>
<td>96.64±0.57 a</td>
<td>1.94±0.23 a</td>
<td>30.28±2.22 abc</td>
<td>0.42±0.003 b</td>
<td>32.00±3.51 b</td>
<td>82.67±28.01 ab</td>
<td>0.74±0.12 bc</td>
</tr>
<tr>
<td></td>
<td>CsA+FO</td>
<td>22.56±4.27 c</td>
<td>1.60±0.24 bc</td>
<td>26.71±2.72 abc</td>
<td>0.43±0.011 a</td>
<td>41.00±3.37 a</td>
<td>44.44±0.32 a</td>
<td>0.65±0.04 a</td>
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<td>19.16±2.82 a</td>
<td>1.85±0.18 bc</td>
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<td>0.43±0.007 a</td>
<td>37.67±4.98 a</td>
<td>39.44±3.70 ab</td>
<td>0.68±0.13 a</td>
</tr>
<tr>
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<td>FO</td>
<td>67.68±2.63 ab</td>
<td>1.43±0.06 c</td>
<td>16.14±0.58 d</td>
<td>0.40±0.011 a</td>
<td>33.50±2.02 a</td>
<td>23.34±4.21 b</td>
<td>0.55±0.03 a</td>
</tr>
<tr>
<td></td>
<td>CsA</td>
<td>57.05±12.15 ab</td>
<td>2.11±0.07 b</td>
<td>28.85±4.36 ab</td>
<td>0.44±0.019 a</td>
<td>44.50±8.37 a</td>
<td>52.59±6.58 a</td>
<td>0.74±0.03 a</td>
</tr>
<tr>
<td></td>
<td>CsA+FO</td>
<td>29.07±7.10 b</td>
<td>1.88±0.07 ab</td>
<td>30.28±0.33 a</td>
<td>0.43±0.012 a</td>
<td>32.33±0.67 bc</td>
<td>61.87±9.82 a</td>
<td>0.74±0.08 bcd</td>
</tr>
<tr>
<td></td>
<td>3rd Week</td>
<td>28.20±6.08 b</td>
<td>2.02±0.16 a</td>
<td>35.28±4.87 a</td>
<td>0.42±0.006 ab</td>
<td>33.33±4.70 bc</td>
<td>68.51±4.65 a</td>
<td>0.81±0.02 abc</td>
</tr>
<tr>
<td></td>
<td>FO</td>
<td>54.88±11.73 a</td>
<td>1.63±0.06 ab</td>
<td>20.71±0.25 b</td>
<td>0.39±0.007 b</td>
<td>27.50±1.44 c</td>
<td>53.33±1.28 ab</td>
<td>0.63±0.02 d</td>
</tr>
<tr>
<td></td>
<td>CsA</td>
<td>51.74±0.94 ab</td>
<td>1.42±0.23 b</td>
<td>29.28±0.58 a</td>
<td>0.41±0.016 ab</td>
<td>31.33±3.84 bc</td>
<td>58.37±3.18 ab</td>
<td>0.68±0.08 cd</td>
</tr>
</tbody>
</table>

(C: Control Normal group, FO: fish oil group, CsA: Cyclosporine A group, CsA+FO: Cyclosporine A + fish oil group). Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P<0.05).
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3. RESULTS

The results presented in (Tables 1 and 2) revealed that CsA-induced nephrotoxicity caused significant increase in serum glucose, lipid profile (total cholesterol, triacylglycerol and phospholipids), renal function tests (urea, uric acid and creatinine), haptoglobin levels, lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) activities with significant decrease in serum total protein, albumin and electrolytes (sodium, potassium and inorganic phosphorus) concentrations. Dietary fish oil administration to CsA treated rats restore serum renal functions tests (urea, uric acid and creatinine), haptoglobin, lipid profiles and serum markers enzyme (LDH and GGT) activities and also reversed the increase in serum proteins and electrolytes to normal range. The obtained results demonstrated in (Table 3) revealed that CsA administration caused significant elevation in kidney tissue malondialdehyde (MDA) along with significant decrease in antioxidant enzymes (CAT, SOD and GPx) activities, non enzymatic antioxidant (GSH), total antioxidant capacity and nitric oxide level in the rat kidney. Meanwhile, dietary fish oil administration to rats received oral dose of CsA improved renal functions by bringing about a significant decrease in peroxidative levels and increase renal tissue antioxidant status as revealed by enhanced renal tissue antioxidant enzymes activities (CAT, SOD and GPx), GSH and total antioxidant capacity levels.

4. DISCUSSION

Nephrotoxicity is the most common and clinically significant adverse effect of cyclosporine [35]. Oxidative stress is the main mechanism resulting in cyclosporine-induced nephrotoxicity as a result of its ability to stimulate endogenous melatonin production [36]. FO prevents gentamicin and cyclosporine-A-induced nephrotoxicity [6]. Cyclosporine treatment to control rats resulted in significant increase in serum glucose concentration compared to control group. Cyclosporine had a direct toxic effect on pancreatic beta cells, whereas a reversible suppression of insulin release has also been documented. Other studies have also demonstrated that greater cyclosporine dosages and trough levels were associated with higher insulin values and indices of insulin resistance (IR) [37]. Cyclosporine belongs to the family of calcineurin inhibitors and acts as a prodrug since it remains inactive until it connects with its cytoplasmic receptor known as cyclophilin [38]. In insulin-secreting cells, calcineurin is involved in the stimulation of insulin gene transcription through the activation of the transcription factor nuclear factor of activated T-cells. Nevertheless, the degree and comparability of the calcineurin inhibitors in impairing beta-cell function is yet to be established [39]. Dietary fish oil administration to CsA treated rats resulted in significant decrease in serum glucose levels compared with CsA-tested group. Feeding Sprague Dawley rats with a fish oil diet for 6 weeks increased the incorporation of n–3 fatty acids into the membrane phospholipid fraction of adipocytes. This was associated with increased insulin sensitivity in the adipocytes, as insulin-stimulated glucose uptake was positively correlated with the degree of unsaturation of membrane phospholipid fatty acids [40]. A significant increase in serum renal functions tests (urea, uric acid and creatinine) concentrations were observed in CsA-treated rats as compared with control group. Chronic administration of CsA for 21 days caused a marked impairment of renal functions along with significant oxidative stress in the kidneys [41]. Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal functions directly by causing renal vasoconstriction or decreasing the glomerular capillary ultrafiltration coefficient; and thus reducing glomerular filtration rate [42]. These findings were
further evident from the marked elevation of serum urea, uric acid, and creatinine concentrations, thereby suggesting a significant functional impairment of kidneys in cyclosporine A-treated rats [35]. Plasma uric acid and creatinine can be used as a rough index of the glomerular filtration rate. High levels of uric acid and creatinine indicate several disturbances in kidney [43]. Dietary fish oil administration to cyclosporine-treated rats exhibited significant decrease in serum renal function (urea, uric acid and creatinine) levels as compared with CsA group. Cyclosporine causes vasoconstriction of afferent and efferent glomerular arterioles, decreased glomerular filtration rate and reduced renal blood flow [44]. The n-3 PUFAs present in fish oil (DHA and EPA) enhance the renal production and excretion of the trienoic series of eicosanoids (PGI\(_3\), PGE\(_3\) and TXA\(_3\)). PGI\(_3\) and PGE\(_3\) are potent renal vasodilators, whereas TXA\(_3\) has little effect on vascular smooth muscle tone. Moreover, production of the dienoic prostaglandins (PGI\(_2\), PGE\(_2\)) and the potent vasoconstrictor TXA\(_2\) is reduced by dietary supplementation with fish oil [45]. Administration of Cyclosporine A to normal rats resulted in significant decrease in serum electrolytes (sodium, potassium) as compared with control group. Cyclosporine A may also exert its cytotoxic effects by altering the activity of different plasma membrane transport systems. Membrane ATPases play a key role in the production and maintenance of gradients that aid in the ion distribution in cells [46]. The decline in the activities of ATPases in cyclosporine A-treated rats may be due to enhanced oxidation of membrane lipids and proteins. Inhibition of membrane bound Na\(^+\),K\(^+\)-ATPase which is present in both basolateral and apical domain of the rat plasma membrane will cause an increase in intracellular Na \(^+\) and loss of K \(^+\) that leads to membrane depolarization. It is well known that cyclosporine A treatment induces a decrease in Na\(^+\), K\(^+\)-ATPase [47] which involves a specific interaction between the drug and the enzyme catalytic subunit [48]. In fact, it was shown in humans and rats that cyclosporine A directly interferes with the membrane permeability of Na \(^+\) and K \(^+\) by disturbing the transmembrane potential and cellular ionic gradients [49]. Increasing evidence supports the point that free radicals are involved in the inactivation of Na\(^+\),K\(^+\)-ATPase and supplementation of antioxidant results in abolishing the inhibitory effect on the enzyme [50]. Administration of dietary fish oil to cyclosporine treated rats induced significant increase in serum electrolytes levels compared with CsA group. The dietary fish liver oil rich in eicosapentanoic and docosahexaenoic fatty acids may prevent the membrane alteration, and by this mechanism prevent the changes in Na\(^+\)/K\(^+\)-ATPase activity [51]. Cyclosporine treatment to normal rats resulted in significant increase in renal tissue (L-MDA) levels as compared with control group. Significant increase in lipid peroxidation during CsA administration which suggests the involvement of oxygen free radicals in the pathogenesis of renal injury. Cyclosporine A treatment has been shown to increase the production of free radicals and the formation of lipid peroxides in vivo and in vitro. Cyclosporine A increased malondialdehyde a stable product of lipid hydroperoxide in isolated hepatic and renal microsomes [52]. An increase in superoxide radical and hydrogen peroxide following CsA has been demonstrated. Moreover, CsA administration results in excess local production of hydroxyl radical, leading to lipid peroxidation and nephrotoxicity [53]. However, administration of dietary fish oil in cyclosporine treated rats significantly decreased renal tissue (L-MDA) levels compared with CsA group. Pretreatment with fish oil (both 5, 10% V/W of diet) there was a significant reduction in the levels of lipid peroxides indicating that fish oil inhibit the lipid oxidation [54]. Oxygen free radical attack objects on the polyunsaturated components of membranes
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and may cause a serious organizational dysfunction within cells and tissues [55]. It has been suggested that the use of omega-3 PUFAs may have ameliorating effect on such damage by two possible ways: First, omega-3 PUFA may increase the levels of catalase within the peroxisome and in the cytoplasm resulting in enhanced defense against free oxygen radicals. Second, omega-3 PUFAs, which has been supplemented may be replaced with Polyunsaturated fatty acid components of the membranes that had been attacked by oxygen free radicals such as superoxide anions, hydrogen peroxide and hydroxyl radicals [56]. High oxidative stress due to hyperglycaemia promotes free radicals generation evidence based mainly on increased lipid peroxidation [57].

Administration of cyclosporine to normal rats exhibited a significant decrease in renal tissue antioxidant enzymes (CAT, SOD and GPx) activities as compared with control group. The presently observed decrease in the catalase activity in CsA-treated rats is due to the decreased availability of NADPH, which is required for catalase activity from its inactive form. Therefore, it is possible that depletion of NADPH production during CsA-treated rats could decrease the catalase activity. Decrease in the activity of GPX during CsA administration indicate the reduction in the level of GSH and increase in the level of peroxides. The depletion of glutathione causes a proportional decrease in H₂O² detoxification by glutathione peroxidase [52]. The decline in renal SOD activity after CsA administration [3]. It is well known that an efficient endogenous antioxidant defense system operates to combat the production of free radicals. The antioxidant enzymes catalase, SOD, GPX and catalase constitute the major defense against ROS-induced oxidative damage. Superoxide dismutase is considered as the first line of defense against the deleterious effects of oxygen radicals in cells, where it scavenges ROS by catalysing the dismutation of superoxide to H₂O² and O². Dietary fish oil administration to cyclosporin-treated rats resulted in a significant increase in renal tissue antioxidant enzymes (CAT, SOD and GPx) activities as compared with CsA group. Under normal physiological conditions a delicate balance exists between the rate of formation of H₂O² via dismutation of O²⁻ by SOD activity and the rate of removal of H₂O² by CAT and glutathione peroxidase. Therefore, any impairment in this pathway will affect the activities of other enzymes in the cascade. N-3 PUFA may stimulate α-tocopherol incorporation into membranes, increasing the level of CAT within both peroxisomes and cytoplasm, resulting in an enhanced defense against reactive oxygen species (ROS) [58]. A significant decrease in renal tissue total antioxidant capacity (TAOC) level was observed in cyclosporine treated normal rats compared with control group. CsA therapy induces overproduction of reactive oxygen species (ROS) in hepatocytes and lowers their antioxidant capacity [59]. However, administration of dietary fish oil to cyclosporine-treated rats caused a significant increase in renal tissue (TAOC) level as compared with CsA group. Fish oil which is rich in n-3PUFAs i.e. EPA and DHA interfere with arachidonic acid cascade by inhibiting 5- lipooxygenase (5-LOX). Incorporation of the n-3 PUFAs with biological membrane, increased antioxidant status normalizes the excited state, controls the physical status of membrane lipids and prevents rises in intracellular Ca in response to oxidative stress [60]. In view of these findings, it is possible to conclude that CsA administration results in pronounced oxidative stress and renal damage. FO treatment significantly ameliorated the renal dysfunction and protected renal function from free radical–mediated injury from CsA by protecting the marker enzymes and further strengthened the antioxidant status of the cell. The results suggest that FO is effective in preventing functional impairment in CsA-induced nephrotoxicity in a rat model.
5. REFERENCES


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التأثير الوقائي للنضج بزيت السمك في التسمم الكلوي المحدث بالسيكلوسبورين في الفئران

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المتخصص العربي

تساعد مضادات الأكسدة في الأغذية، على حماية الجسم من العديد من الأمراض المتنوعة، وتستعمل تلك الأمراض السرطان وأمراض الكبد وأمراض الحساسية وأمراض القلب وأمراض الكلى وغيرهما من الأمراض الضارة، أما الجذور الحرة فهي تتأثر بسبب انتفاخ الخلايا، ومن ضمنها الخلايا الكلى، مما يساهم في التسمم الكلوي ويقوم الجسم عادةً بصنع إنزيمات تسمى إنزيمات تمشي بتعديل الجذور الحرة، بالإضافة لهذه الأطروحات.


وخلصت الدراسة على أن التسمم الكلوي له تأثير ضار على المكونات الكيميائية الحيوية للسيكلوسبورين، وعندما يتم إعطاءه إلى الخلايا، يستخدم سيكلوسبورين ذات الثلاجة لزراعة الخلايا، بل ويؤدي إلى نتائج الضارة، وأدى إلى انتفاخ الخلايا، وتم تشتيت الخلايا حسب النتائج، بالإضافة إلى إنتاج الخلايا الكبيرة، وبالتالي يمكن أن تؤدي النتائج الضارة إلى نتائج بسيطة في ظل الخلايا التي تنتج عند حصول الجذور الحرة.

للمزيد من فضائل البسالة والدقة الطبية السريعة للدروز الضارة، فقد اقترح أن التسمم الكلوي يمكن أن يكون من فضائل البسالة والدقة الطبية السريعة للدروز الضارة.