Garlic oil as an effective protective agent against cyclosporine-induced nephrotoxicity in rats

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Abstract: In this work, thirty adult male albino rats were used to study the possible beneficial effect of garlic oil (GO) in preventing renal injury and related oxidative stress caused by administration of cyclosporine (CsA) in rats in an attempt to understand its mechanism of action. Rats were divided into three groups having 10 rats in each. Group I was given olive oil; group II was given CsA (25 mg/kg body weight daily for 3 weeks); and group III were given GO orally (5 ml/kg body weight) 30 min before CsA administration for 3 weeks. At the end of the experimental period blood Urea Nitrogen (BUN), creatinine, fractional excretion of sodium (FENa), microalbumin excretion, total protein and N-acetyl-β-glucosaminidase were determined, while the glomerular filtration rate (GFR) was estimated by creatinine clearance. Malondialdehyde (MDA) level was used as biomarker for testing the antioxidant potential of the drug as well as the changes in serum nitric oxide (NO) levels. The study showed that CsA induced oxidative stress, as indicated by increased plasma levels of MDA and serum NO levels, and deteriorated the renal function as assessed by increased serum BUN, creatinine, microalbumin, FENa and NAG while the total protein and the GFR was significantly lower compared with control rats. GO significantly attenuated renal dysfunction and the reduction in serum NO levels and counteracted the deleterious effects of CsA on MDA while the GFR was significantly high. In addition, fractional excretion of sodium and NAG were significantly inhibited following GO administration. Hence, GO has a strong potential to be used as a therapeutic adjuvant in CsA nephrotoxicity.

Keywords: cyclosporine; nephrotoxicity; reactive oxygen species; renoprotective.

Introduction

Cyclosporine-A is the most common immunosuppressive drug used in inflammatory disorders, autoimmune diseases and organ transplantation. Nephrotoxicity is the major limitation of CsA use (Capasso et al., 2007). CsA induces lipid peroxidation, NO production (Amore et al., 2000), reduces renal NADPH cytochrome P450 and reduced/oxidized glutathione ratio as well as microsomes and mitochondria in the kidneys (Rezzani, 2006).

A member of the Liliaceae family, garlic (Allium sativum) is a commonly worldwide used food, and its medical properties have been well recognized since the ancient times (Tattelman, 2005). Ancient Egyptian records mentioned that use of garlic as a remedy for a variety of diseases. These pharmacological effects of garlic are attributed to the presence of pharmacologically active sulfur compounds including diallyl sulfide, diallyl disulfide, allicin, and dipropyl sulfide. These compounds have been known to increase the activity of enzymes involved in the metabolism of carcinogens (Galeone et al., 2008 and Shukla and Kalra, 2007). In addition, GO is hypolipidemic (Mahmoodi et al., 2006) hypoglycemic (El-Demerdash et al., 2005), antiatherosclerotic (Koscielny et al., 1999 and Siegel and Klüssendorf, 2000) properties, anti-hypertensive (Silagy and Neil, 1994), anticoagulant (Chan et al., 2007), anti-hepatic injury (Sabry et al., 2009) and an antioxidant against free radicals (Jabbari et al., 2005 and Morihara et al., 2006). Chronic administration of aqueous garlic homogenate reduced lipid peroxidation in rat liver and kidney (Banerjee et al., 2001). However, the protective effect of garlic against CsA nephrotoxicity remains obscure. Therefore, the present study was undertaken to evaluate the ability of GO administration to alleviate the adverse effects of CsA on the kidney.
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Materials and methods

Chemicals

Garlic (*Allium sativum*) belongs to family Alliaceae was considered one of the most important plants that has been used for decades by the Egyptian population. Garlic oil was purchased from El-Captain Company (Cairo, Egypt). GO was given by gavage at a dose of 5 ml/kg as described by Hassan et al. (2009). Cs A was obtained from Novartis Pharma AG, Basle, Switzerland. Cs A was dissolved in olive oil so as to give a final concentration of 25 mg / 2 ml (Tariq et al., 1999).

Animals

Thirty male albino rats weighing 150-180 gm were used in this study purchased from Helwan Breeding Farm, and left in our laboratory for 2 week before beginning the experiment for acclimatization. The animals were kept under good ventilation and received a balanced diet and water ad libitum throughout the experimental period. They were kept at 22–24 °C with the 12 h light/dark cycle and received human care. The animals were randomly divided into three groups having 10 rats in each. Group I served as a control, whereas groups II and III were used as experimental. Group I was given olive oil; group II was given Cs A orally at a dose of 25 mg/kg body weight daily for 3 weeks; and group III were given orally similar dose of Cs A as group II after 30 min of GO administration orally (5 ml/kg body weight) for 3 weeks.

Blood sampling

At the end of the experiment, blood was withdrawn from the retrobulbar venous plexus under ether anesthesia. Samples were collected in clean, dry and graduated centrifuge tubes, which were left for 15 minutes to clot, and then centrifuged at 3000 rpm for 15 minutes to separate the serum. They were kept at 20 °C for biochemical analysis.

Biochemical assays

Plasma samples were assayed for: blood urea nitrogen (BUN) was measured colorimetrically at wavelength 578 nm (Newman and Price, 2001) and Creatinine (Cr) was measured at wavelength 492 nm (Newman and Price, 2001). Urine samples were stored at -20°C to avoid loss of enzyme activity and for the determination of fractional excretion of sodium, creatinine and microalbumin. Urinary NAG was determined colorimetrically according to the method of Moore and Morris (1982).

Detection of MAD and nitric oxide

Peroxidation was measured as the production of malondialdehyde (MDA), which in combination with thiobarbituric acid (TBA) forms a pink chromogen compound whose absorbance at 532 nm was recorded (Stocks and Dormandy, 1971). Plasma nitric oxide was determined as nitrate concentration after reduction of nitrate to nitrite by nitrate reductase. The reaction was performed at 22 °C for 20 min (Moshage et al., 1995).

Detection of fractional excretion of sodium (FENa)

FENa is a measure of the percentage of sodium excreted in the urine versus the sodium reabsorbed by the kidney. It is measured in terms of plasma and urine sodium, rather than by the interpretation of urinary sodium concentration alone, as urinary sodium concentrations can vary with water resorption. This is calculated by the standard equation described by Steiner (1984).

Glomerular filtration rate assessment

Renal function was assessed by at the end of the experimental treatment colorimetric assay of urinary creatinine. Creatinine clearance was calculated using standard formulae according to the method of Wetzels et al. (1988).

Statistical analysis

Statistical analysis was carried out using student “t” test. The results were expressed as mean ± S.D. (N=10). P<0.05 was accepted as the level of significance.
**Results**

Table 1 shows the effect of GO on the renal functions among the different groups. Cs A administration deteriorated the renal function as assessed by significant increased serum BUN (53.61±6.38) and creatinine (2.82±0.86). Cs A produced significant elevated levels of serum MDA (19.18±1.40) and NO (49.23±5.39). All the previous changes were significantly different from the corresponding values in the control group. GO significantly attenuated renal dysfunction in Cs A treated rats as assessed by decreased serum BUN (25.65±2.57) (Figure 1) and creatinine (1.23±0.34) (Figure 2). GO administration with Cs A produced significant decrease in serum MDA level (13.07±0.98) as well as in serum NO levels (40.97±4.30) (Figure 5) as compared to CA-treated group for the previous tests, respectively.

Table 2 shows the effect of GO on body weight, microalbumin, total protein, FENa, NAG and creatinine clearance in Cs A-treated rats. Cs A caused a significant decrease in body weight gain (136 ±16.87) as compared with control group (162 ±11.31), however, GO treatment significantly attenuated Cs A-induced decrease in body weight (152 ±12.35). In addition, Cs A had elevated urinary microalbumin excretion (20.52±2.49), FENa (2.61 ± 0.73) and NAG levels (22.38±2.38) (Figure 3) that were markedly decreased by GO (12.72±0.84, 0.68 ± 0.21 and 7.67±1.56) for the previous tests, respectively. GO induced elevation in total protein (49.11±2.22) and creatinine clearance (2.73 ±0.67) (Figure 4) as compared with Cs A-treated group (35.51±1.89) and 1.09 ± 0.32) respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>Cs A -treated group</th>
<th>Cs A + GO- treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>162 ±11.31</td>
<td>136 ±16.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>152 ±12.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microalbumin (mg/L)</td>
<td>11.19±1.13</td>
<td>20.52±2.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.72±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>53.72±3.36</td>
<td>35.51±1.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.11±2.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.49 ± 0.19</td>
<td>2.61 ± 0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary NAG (U/g creatinine)</td>
<td>5.36±0.89</td>
<td>22.38±2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.67±1.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>3.71±0.69</td>
<td>1.09 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> compared with the control group.
<sup>b</sup> compared with the Cs A-treated group.
* P < 0.05

![Figure 1](http://www.openaccessscience.com)

**Figure 1:** Effects of GO on serum BUN in Cs A-treated rats.

![Figure 2](http://www.openaccessscience.com)

**Figure 2:** Effects of GO on serum Cr in Cs A-treated rats.
Discussion

Treatment of rats with Cs A for a period of 3 weeks resulted in nephrotoxicity that was characterized by a decline in kidney function, increase of serum BUN, creatinine, fractional excretion of Na, urinary N-acetyl-β-glucosaminidase excretion (FENa and NAG, a markers of renal tubular injury) and increase in microalbumin excretion (an index of glomerular capillary damage). The mechanisms of Cs A-induced nephrotoxicity are not fully elucidated, but several researchers suggest that a defect in intracellular calcium handling (Cheng et al., 2002), generation of reactive oxygen species (ROS) as a result of renal vasoconstriction with subsequent hypoxia reoxygenation injury (Tirkey et al., 2005), and nitric oxide (NO) system are involved (De Nicola et al., 1993). Cs A was reported to reduce food intake in rats and reduction in protein intake is known to exert a marked suppressive effect on GFR (Wongmekiart and Thamprasert, 2005).

The nephroprotective effect of GO is evident by improvement of renal function, as judged by significant decreases in levels of BUN (Figure 1) and creatinine (Figure 2) and these results were in accordance with that of Gulnaz et al. (2010). In addition, GO treatment directly protects Cs A general toxicity as evidenced by significant prevention of weight loss. GO was able to prevent the decrease of GFR through its action on the arteriolopathy. Arteriolopathy is considered one of the major causes of reduced GFR following Cs A administration (Capasso et al., 2007).

On the other hand, GO administration resulted in increase of serum total protein (Table 2) by preventing protein oxidation (Metwally, 2009). This effect probably reflects the potential ability of GO to protect the protein manufacturing machinery from NaNO₂ induced cellular damage and increasing the immunoglobulin and total globulin concentrations (Shalaby et al., 2006). Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. High serum protein levels have been reported due to improve liver and other organs.
functions which synthesized plasma protein (Hussein et al., 2001).

The presence of glomerular damage in Cs A treated group was reflected by the increase of urinary microalbumin excretion (Table 2). Glomerular damage is presumably due to transient impairment of the glomerular capillary permeability (Brezis and Rosen, 1995) and a reduction in tubular reabsorption of filtered albumin further contributes to the urinary microalbumin excretion (Eppel et al., 1999). GO treatment was accompanied by significant reduction in urinary excretion of microalbumin. GO may influence the integrity of the glomerular basement membrane and retard the abnormal passage of high molecular weight molecules from the membrane. In addition, GO might play a critical role in suppressing ROS mediated destruction of basement membrane and proteinuria (Anwar and Meki, 2003).

Tubular cell damage in Cs A treated group was evidenced by a significant decrease in tubular sodium reabsorption and increase FENa, and a significant increase in urinary excretion of NAG. Daily intake of GO record significant decrease in creatinine clearance (Figure 4), urinary levels of FENa and NAG (Figure 3) supports the idea that GO exhibits potent anti-inflammatory and membrane stabilizing properties (Ban et al., 2009) and thus prevents exposure to proinflammatory cytokines that induce the damage of the tubular epithelial cells in Cs A rats (Ban et al., 2007).

The present study revealed that; GO administration resulted in reduction of serum NO and MDA levels (Figure 5). The antioxidant action of GO can be explained by scavenging or neutralizing of free radicals, inhibiting hydrogen peroxide and tumor necrosis factor – alpha (Ban et al., 2007), inhibiting xanthine oxide (Ou et al. 2003), interacting with oxidative cascade and preventing its outcome, oxygen quenching and making it less available for oxidative reaction, inhibition of cytochrome P450 (HO et al., 2010). However, GO improved the antioxidant mechanism due to the ability of Dialyl disulfide and Diallyl trisulfide present in GO in modulating the oxidative stress and detoxifying enzyme system (Pedraza-Chaverrí et al., 2003 and Hassan et al., 2009). They reduce pro-inflammatory cytokines through blockade of nuclear factor kappa-B (NF-kB) (Ban et al., 2007). Furthermore, GO reduce ischemic injury and improvement of renal blood flow of Cs A-treated rats (Bagheri et al., 2011) and blockade of cell mediated cytotoxicity (Oommen et al., 2004). In addition, GO possibly prevent the reactive oxygen species from acting on DNA and production of DNA fragments and micronuclei formation (Zhang at al., 2012), suggesting a possible role of GO as a chain breaking antioxidant against lipid peroxidation (Pari et., 2007). GO inhibits formation of nitrite by competing with the oxygen atom to react with NO (Pedraza-Chaverrí et al., 2004), inhibits the redox-active transcription factors required for iNOS induction and reduces NO oxidation by sequestering the reaction intermediate, nitrogen dioxide (Hassan et al., 2010).

Conclusion

Co-administration of GO during Cs A therapy has a significant renoprotective effect in a rat model of Cs A-induced renal injury, thus proving itself as an effective antioxidant and membrane stabilizing. However, if relevant in humans, this finding may be of a major importance, introducing a safe, inexpensive and feasible method for attenuation of Cs A-induced nephrotoxicity.

References


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