Effect of curcumin on intra-peritoneal glycerol induced acute renal failure in rats

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ABSTRACT

Glycerol is used for treating brain edema. The potential toxicity increases in glycerol kinase deficiency is associated with glycerol levels in plasma and urine that are more than 100-fold higher than normal. The present study aims to investigate the protective effects of curcumin on glycerol induced acute renal failure using a model of glycerol intra-peritoneal administration with 24 h dehydration in albino rats. Twenty-four hours after the administration of glycerol, all rats were sacrificed. Urea and creatinine levels were measured in the blood, and the levels of malondialdehyde, superoxide dismutase and glutathione-S-transferase activity in renal homogenate were determined in the renal tissue. Glycerol administration resulted in elevation in levels of renal malondialdehyde in addition to reduction of superoxide dismutase and glutathione-S-transferase activity and deterioration in the renal functions as assesseed by the increased in plasma urea and creatinine as compared to control rats. Curcumin in a dose of 200-mg/kg body weights for 30 min after the injection of glycerol significantly reduced the elevated malondialdehyde
levels and increased the antioxidant enzyme activity in rats treated with intra-peritoneal glycerol. In conclusion curcumin might be a potential candidate agent against glycerol-induced nephrotoxicity.

Key Words: Curcumin, glycerol, renal failure, antioxidants enzymes.

INTRODUCTION

Glycerol is a chemical compound with three hydrophilic alcohol hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. Glycerol constitutes the main osmoprotective solute and has been used to improve physical endurance. The ingestion of glycerol and liquid is used to increase body water volume by reducing the rate at which the kidneys eliminate water, thus maintaining longer hydration. Glycerol is used in the treatment of cerebral oedema, glaucoma and increase intracranial pressure because it increases blood osmolarity, causing a decrease in aqueous volume and thus reducing pressure and edema (Brisson et al., 2001).

Experimentally, intramuscular administration of hypertonic glycerol solution was used to produce acute renal failure (Giannoglou et al., 2007). In this model, glycerol causes rhabdomyolysis and myoglobinuria, resulting in the development of renal injury. The pathogenesis is thought to involve vascular congestion, formation of casts and oxidative stress (Lieberthal and Nigam, 2000). On the other hand the intra-peritoneal administration of 10 ml/kg, hypertonic glycerol solution (50% v/v) followed by 24 h water deprivation did not alter plasma creatine kinase (CK) activity and increased plasma
creatinine levels, suggesting renal insufficiency and absence of rhabdomyolysis. Also, renal CK and pyruvate kinase activity was decreased, suggesting diminution of energy homeostasis in the kidney. Plasma and renal lactate dehydrogenase (LDH) activity was decreased, whereas the formation of free radicals, lipid peroxidation and protein carbonylation were increased, suggesting oxidative stress. These results are similar to those described after the intramuscular administration of glycerol (Aydogdu et al., 2006). Therefore, it is possible that glycerol may provoke renal lesions by mechanisms other than those induced by rhabdomyolysis (Rieger et al., 2008). These mechanisms include free radicals, lipid peroxidation, iron accumulation in the kidney, depletion of the anti-oxidant status, glomerular dysfunction, decreased NO levels and impaired renal morphology (Ayodu et al., 2006).

Oxidative stress is counteracted in biological systems by a large set of endogenous antioxidants, including enzymes such as superoxide dismutases, catalase, and glutathione peroxidase, as well as low molecular weight compounds, such as glutathione, generally found at levels sufficient enough to defend cells from oxidative insult (Floyd, 1999).

Curcumin is a major yellow pigment in turmeric (the ground rhizome of Curcuma longa Linn.), which is widely used as a spice and a coloring agent in several foods such as curry, mustard and potato chips as well as in cosmetics and drugs (Okada et al., 2001; Joe et al., 2004).
Recently, curcumin is recognized as a promising compound with multiple pharmacological properties including anti-inflammatory, anticarcinogenic, and anti-thrombotic (Srimal, 1997; Shukla et al., 2008). Curcumin is unique over other natural antioxidants since it possesses both the phenolic and diketonic groups, which help in the scavenging of free radicals. In contrast, other natural antioxidants possess either phenolic or diketonic groups (Srimal, 1997). Moreover curcumin is highly lipophilic and might cross the BBB and reach the brain, but its bioavailability is very low, since the drug is rapidly metabolized by conjugation (Kelloff et al., 1996)

**MATERIALS AND METHODS**

**Chemicals**

Chemicals required for all biochemical assays were analytical grade obtained from Sigma-Aldrich Chemicals Co., USA.

**Animals**

Male albino rats weighing 150-200 g were used in this study. The rats were housed for one week for acclimatization in clean plastic cages in a temperature- and humidity-controlled facility with a constant 12 h light/dark cycle. Food was offered daily during the study period. The rats described below comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by the National Egyptian Community.

**Treatment and experimental design**
After one week of acclimatization in cages, the rats were randomly divided into four groups, each consisting of six rats.

**Group 1:** rats served as control and received a single dose of i.p. injection of 1 ml isotonic saline.

**Group 2:** rats received 10 ml /kg, i.p, hypertonic glycerol solution (50% v/v) followed by 24 h water deprivation *(Rieger et al., 2008).*

**Group 3:** glycerol/dehydration rats received curcumin 30 min after the injection of glycerol. Curcumin was dissolved in corn oil and was injected i.p at the dose of 200-mg/kg body weights *(Chuang et al., 2000).*

**Group 4:** glycerol/dehydration rats received corn oil (the curcumin vehicles). 30 min after the injection of glycerol.

Twenty-four hours after the administration of glycerol, blood was collected from retro-orbital sinus and centrifuged for serum separation for determination of creatinine and urea. Animals were sacrificed, then kidneys were rapidly excised, after weighing the kidney tissue, homogenate, supernatant samples were prepared for biochemical parameters determination (SOD, GST activity and lipid peroxides level).

**Determination of serum creatinine and urea**

Creatinine reacts with alkaline picrate to form an amber yellow color that is measured spectrophoto-metrically *(Higgines, 1995).*
Colorimetric determination of urea depend on the activity of urease to give ammonium ion which react with salicylate and hypochlorite to form a green color (Patton and Crouch, 1977).

Evaluation of antioxidant enzymes

After decapitation of all rats, their kidneys were removed, weighed and homogenized for evolution of antioxidant enzymes.

Determination of lipid peroxides level

Thiobarbituric acid reactive substances (TBARS) was measured spectrophotometrically using thiobarbituric acid (Uchiyama and Mihara, 1978).

Determination of glutathione-S-transferase (GST) activity

The GST activity toward 1-chloro-2,4-dinitrobenzene in presence of glutathione as co-substrate was examined spectrophotometrically at 25°C. The enzyme activity was determined by monitoring change in absorbance at 340 nm. (Habig et al., 1974)

Determination of superoxide dismutase (SOD) activity

SOD activity was determined by the difference between autooxidation of pyrogallol alone and in presence of homogenate that contain SOD (Marklund, 1985).
Statistics

The statistical significance of the differences were calculated using one way analysis of variance (ANOVA) followed by the Bonferroni test for multiple comparisons.

RESULTS

Intraperitoneal glycerol administration caused acute renal failure (increased serum urea and creatinine levels significantly (\( P < 0.05 \)) as shown in table-1 and a marked renal oxidative stress (increased lipid peroxidation in addition to decreased SOD and glutathione S transferase significantly, \( P < 0.05 \) (Table 2). vehicle did not induce significant changes in the measured parameters. (Table1, 2)

Curcumin in a dose of 200 mg/d IP improve both renal function parameters (serum urea and creatinine) and oxidative stress parameters (lipid peroxidation, SOD and Glutathione - S- transferase) significantly, \( P<0.05 \) (table 1,2)

Table (1): Effect of curcumin (200 mg/kg, i.p) on serum creatinine and urea

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 2 (Glycerol/dehydration)</th>
<th>Group 3 (Glycerol/dehydration + curcumin)</th>
<th>Group 4 (Curcumin vehicle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea</td>
<td>23.5</td>
<td>69.4</td>
<td>28.5*</td>
<td>23.9</td>
</tr>
</tbody>
</table>
Serum creatinine (mg/dl)  

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 2 (Glycerol/dehydration)</th>
<th>Group 3 (Glycerol/dehydration + curcumin)</th>
<th>Group 4 (Curcumin vehicle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super oxide dismutase (U/mg protein)</td>
<td>16.4 ± 0.06</td>
<td>8.24 ± 0.07^</td>
<td>13.5 ± 0.04^</td>
<td>16.8 ± 0.07</td>
</tr>
<tr>
<td>Glutathione-S-transferase (nmol/min/mg protein)</td>
<td>8.77 ± 1.6</td>
<td>5.1 ± 0.8^</td>
<td>7.39 ± 0.56^</td>
<td>8.79 ± 1.6</td>
</tr>
<tr>
<td>TBARs</td>
<td>55.3</td>
<td>135.6</td>
<td>75.3</td>
<td>58.9</td>
</tr>
</tbody>
</table>

+ Significant difference (P<0.05) compared to control group

* Significant difference (P<0.05) compared to Glycerol/dehydration group.

**Table (2):** Effect of curcumin (200 mg/kg, i.p) superoxide dismutase, glutathione-S-transferase and lipid peroxides level in rats renal homogenate.
| (nmol/ gm tissue) | ± 5.2 | ± 8.2* | ± 5.8* | ± 5.1 |

+ = Significant difference (P<0,05) compared to control group

* = Significant difference (P<0,05) compared to Glycerol/dehydration group.

**DISCUSSION**

Glycerol has been used at doses up to 2 g/kg body weight to treat high intracranial pressure, cerebral oedema and glaucoma, as well as to increase the body resistance of athletes (Frank et al., 1981). Glycerol kinase deficiency is associated with increase in glycerol levels in plasma and urine more than 100-fold higher than normal (McCabe, 2001, Kaal and Vecht, 2004)

The intramuscular administration of hypertonic glycerol solution was used to produce acute renal failure (Giannoglou et al., 2007). This model is not simulating the possible clinical toxicity of glycerol as rhabdomyolysis and myoglobinuria play a role in the development of renal injury. The pathogenesis is thought to involve vascular congestion, the formation of casts (Lieberthal and Nigam, 2000).

The model of intra-peritoneal glycerol induced acute renal failure is closely similar to the possible clinical toxicity of glycerol. (Rieger et al., 2008)

The present work is in agreement with the results of Rieger et al. (2008) who showed that intraperitoneal glycerol injection could cause acute renal failure and renal oxidative stress. The reduction of SOD and glutathione -S- transferase were added by this work to
the oxidative changes induced by intraperitoneal glycerol injection reported by Rieger et al. (2008).

Moreover, the present work suggested that curcumin could cause nephroprotection against glycerol induced acute renal failure through improvement in the antioxidant enzymes. These results are in agreement with recent documents, which reported the nephroprotective effect of Curcumin.

Bayrak et al. (2008) concluded that Curcumin significantly improved the urea and cystatin C levels in ischemia-reperfusion (I/R) treated with curcumin group compared to I/R group. Reduction of serum glutathione peroxidase was significantly improved by curcumin. Treatment with curcumin also resulted in significant decrease in serum and tissue malondialdehyde, nitric oxide and protein carbonyl and for tissue that were increased by renal I/R injury. In histological examination, the rats treated with curcumin had nearly normal morphology of the kidney.

Osawa (2007) concluded that curcumin has been reported to have nephroprotective effect as it improved creatinine and urea clearance and also protected the chronic renal allograft nephropathy. These beneficial effects have been explained by alleviation the oxidative stress and the induction of antioxidative enzymes.

In conclusion Curcumin might be a potential candidate agent against glycerol-induced acute renal failure through its antioxidant effects.
REFERENCES


