Ocimum gratissimum aqueous extract enhances recovery in cisplatin - induced nephrotoxicity in albino Wistar rats

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Abstract
In the current study, the dose dependent (5% and 10%) and time course curative potential of aqueous leaf extract of Ocimum gratissimum (O.G.) on cisplatin induced nephrotic rats using biochemical and histopathological approaches was evaluated. Male albino wistar rats weighing between 150-200g were randomly separated into four different groups. Tissue damage was induced in rats of groups 2, 3 and 4 by a single intraperitoneal administration of cisplatin (5mg/kg b.w). Test rats in groups’ 3 and 4 were treated 3 days after cisplatin injection intraperitoneally (i.p) with 5% and 10% C.C accordingly for 3, 6, 9 and 12 days. Rats in group 2 were given sterile water in place of the extracts while rats in group I were the untreated controls. They were all allowed unlimited access to tap water and growers’ mash. Cisplatin treatment caused increases (P ≤ 0.05) in serum urea from 5.733 ± 0.06 to 13.000 ± 0.10 mmol/l, creatinine, uric acid, urine volume and urinary protein. Significant decreases (P ≤ 0.05) in urinary creatinine were also observed. There were considerable decreases (P ≤ 0.05) in body weight and increase (P≤ 0.05) in kidney weight to body weight ratio. However most of these changes were alleviated by prophylactic treatment with aqueous extract of Ocimum gratissimum dose and time dependently (P ≤ 0.05).The ameliorating effect was further evident through decreased histopathological alterations of kidney tissues in the groups treated with aqueous extract of Ocimum gratissimum (5% and 10%). The results of this study indicate that aqueous leaf extracts of Ocimum gratissimum affect the course of tubular repair after the onset of cisplatin-induced nephrotoxicity in rats with accelerated recovery. Hence the extracts have the potential to be used for the management of nephropathies and as a therapeutic adjuvant in cisplatin toxicity.

Key words Ocimum gratissimum nephrotoxicity cisplatin nephropathies

Introduction
Investigations into the chemical and biological activities of plants during the past two centuries have yielded components for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja & Rao, 2000). Plant foods especially vegetables contribute to both local diets and ethno medicine in developing countries like Nigeria (Okafor, 1980, Gbile & Adesina, 1986). Ocimum gratissimum (Linn), family labiaceae is a shrub commonly found around village huts and gardens (Iwu, 1993).

The plant is popular among various ethnic groups in Nigeria. It is known as effin ajasin in Yoruba, ebavbokho in Bini, aaid dya ta gida in Hausa, nchanwu in Ibo, froukena in Ijaw and oran in Urhobo. Mostly a weed of the road sides and wasteland, but is also important in pastures. It prefers moist and fertile soils during growth, but will tolerate drought after flowering (Swabirk, 1997). The plant occurs in deciduous forests and savannah and is usually cultivated for its medicinal uses and as food flavour. Ocimum gratissimum (O.G) is propagated by seeds or cuttings.

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it is used for medicinal, condiment and culinary purposes. The flowers and leaves of this plant are rich in essential oils, and so it is used in preparation of teas and infusion (Rabelo et al., 2003). In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea (Effraim et al., 2003). In the Savannah areas decoctions of the leaves are
used to treat mental illness (Akinmoladun et al., 2007).

*O. gratissimum* is used by the Ibo's of South Eastern Nigeria in the management of the baby's cord; to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh et al., 2005). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye and skin, pneumonia, cough, fever and conjunctivitis (Adebola and Salau, 2005). The oil is known to exhibit antimicrobial, insect repellant, and antihelmintic activities (Sofowora, 1982). Oboh (2008) reported the antioxidant properties of *O. gratissimum*. The extract of *O. gratissimum* exhibited antibacterial activity (Ofokansi et al., 2003). This extract when fed to rabbits reduced the weight and suppressed the hemopoietic system (Effraim et al., 2000). There is also report that leaf extracts from the plant are able to inhibit and even reverse carbon tetrachloride induced hepatotoxicity in rats (Arhogho et al., 2009).

Obianime et al., (2011) reported that the serum levels of the hepatic enzymes on prolonged administration of O.G in mouse were not significantly altered. This suggests that hepatic function in the mouse is not adversely affected by O.G. Aguiyi et al., (2000) also reported the hypoglycaemic activity of *O. gratissimum*. The incidence of kidney failure or chronic kidney failure has doubled over the last 15 years. It is estimated that currently, there are over one million people worldwide who are alive on dialysis or with functioning graft. Patients who suffer from kidney disease are not able to afford the cost of treatment. The crisis of kidney shortage is a global phenomenon and it is worst in developing countries. Many herbal drugs have been investigated for various ailments, however, very few have been considered for nephrocurative activity. In this present study we report the dose and time dependent effects of the aqueous leaf extracts of *O. gratissimum* on cisplatin induced kidney dysfunction.

**Materials and methods**

**Animals**

Seventy two (72) adult healthy male wistar albino rats, weighing between 150 and 200 g were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

**Drugs and chemicals**

Cisplatin was a product of Korea United Pharm INC, KOREA. Kits from Teco diagnostics Ltd. USA, HUMAN diagnostics Ltd. Germany, Fortress diagnostics Ltd. United Kingdom were used. All other reagents/chemicals obtained from standard suppliers were of analytical grade.

**Preparation of extracts**

The leaves of *O. gratissimum* were collected from Sagbama in Bayelsa State of Nigeria and were identified at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Air dried leaves of *O. gratissimum* were grounded and later milled into powder form. 50g portion of the milled leaf was weighed and soaked in 500ml of distilled water in a beaker. The mixture was shaken and kept on the laboratory bench for 24hrs before filtering. The filtrate was evaporated to dryness at room temperature in a rotary evaporator to obtain a paste which was further dried in a dessicator with constant changing of the self-indicating silica gel. Appropriate weights of the residue were prepared in distilled water to obtain concentrations of 5% and 10% (w/v) of O. gratissimum that were administered orally to each of the rats.

**Experimental design and procedures,**
Cisplatin model for evaluation of antinephrotoxic activity, Cisplatin BP (50mg/50ml) was administered to the test rats intraperitoneally at a dose of 5mg/Kg body weight (Mansour et al., 2006; Okoko and Oruambo, 2008).

Evaluation of curative potential, The rats were divided into four equal groups of eighteen (18) rats per group. In-group 1 the rats received no cisplatin. Normal saline was administered i.p. The second group was injected with a single dose of cisplatin (5 mg/kg, i.p) at the beginning of the experiment (Mansour et al., 2006).

Tissue damage was also induced in rats in groups 3 and 4 by a single intraperitoneal-administration of cisplatin (5 mg/kg body weight). Three days later, 2ml/kg body weight of 5% and 10% aqueous extract of Ocimum gratissimum were administered to rats in groups 3 and 4 respectively through the oral route using the gavage once daily for 3, 6, 9 and 12 days. Rats in group 2 were given sterile water in place of the extracts. Rats in-group 1 were untreated controls. They were all allowed unlimited access to tap water and growers’ mash. During the experimental period, animal behavior and body weights were recorded daily. Randomly selected animals of different groups were anaesthetized with urethane. Blood samples were collected by cardiac puncture after 0, 3, 6, 9, 12 and 15 days for biochemical analyses. Parts of the liver tissues were immediately taken and fixed in 10% neutral buffered formalin for histopathologic examination.

Kidney as ratio of body weight, kidney was removed and weighed immediately. kidney ratio was calculated with the following formula, organ ratio(%) = organ weight(g) X 100/body weight(g)

Biochemical Analysis, After the experimental period, animals in different groups were sacrificed. Blood was collected in tubes without anticoagulant to separate serum for various biochemical estimations.

Renal markers, Serum urea was assayed by the modified Berthelot method according to Tobacco et al (1979). Creatinine was determined by the colorimetric kinetic method by Bartels et al. (1971). Serum uric acid was estimated using the enzymatic colorimetric method employing uricase according to Duncan et al. (1982). Albumin was estimated by the Bromocresol green method according to Doumas et al. (1971). Urinary protein was estimated using the Biuret method (Tietz, 1983).

Histopathological study, Small pieces of kidney tissues were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6μm in thickness were cut and stained with haematoxylin and eosin.

Statistical analysis
Data was expressed as Mean ± SD of three estimations. The statistical significance was evaluated by ANOVA using SPSS Version 16 and the individual comparison were obtained by LSD and Tukey method. Values were considered statistically significant when P < 0.05. In order to discern the possible interaction between cisplatin and Ocimum gratissimum, two-way analysis of variance was used.

Results
Animal model of cisplatin induced nephrotoxicity was used for the present study. There was significant (P≤0.05) decrease in body weight in the cisplatin treated rats after 15 days when compared with the normal (control) rats (Table 1).

The body weights of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of Ocimum gratissimum, in groups 3 and 4 respectively, decreased significantly on the 3rd and 6th day but increased on the 9th, 12th and 15th day when compared to the cisplatin treated group (P≤0.05) (Table 1).

The increase of the body weights by the extract though not statistically significant (P≥0.05) was dose dependent. Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on body weight (P≤0.05) (Table 1).
Table 1. Effect of administration of aqueous extract of O.g on body weight (g) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days¹</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (normal saline)</td>
<td>165.93 ± 0.31*</td>
<td>174.33 ± 0.29*</td>
<td>175.73 ± 0.46*</td>
<td>179.43 ± 0.40*</td>
<td>190.50 ± 0.50*</td>
<td>209.17 ± 0.29*</td>
</tr>
<tr>
<td>2. Cis (5mg/kg i.p)+2ml O.G</td>
<td>215.50 ± 0.50*</td>
<td>162.83 ± 0.29*</td>
<td>155.50 ± 0.50*</td>
<td>159.77 ± 0.25*</td>
<td>162.93 ± 0.31*</td>
<td>159.97 ± 0.87*</td>
</tr>
<tr>
<td>3. Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>176.93 ± 0.31*</td>
<td>153.90 ± 0.26*</td>
<td>149.97 ± 0.15*</td>
<td>156.73 ± 0.31*</td>
<td>164.13 ± 0.15*</td>
<td>175.17 ± 0.40*</td>
</tr>
<tr>
<td>4. Cis (5mg/kg i.p)+2ml 10% O.G</td>
<td>164.10 ± 0.36*</td>
<td>137.10 ± 0.36*</td>
<td>140.67 ± 0.58*</td>
<td>149.80 ± 0.26*</td>
<td>155.87 ± 0.42*</td>
<td>162.90 ± 0.17*</td>
</tr>
<tr>
<td>Results of one-way ANOVA F-value; P-value</td>
<td>14250; p&lt; 0.05</td>
<td>11270; p&lt; 0.05</td>
<td>4110; p&lt; 0.05</td>
<td>4206; p&lt; 0.05</td>
<td>7010; p&lt; 0.05</td>
<td>7011; p&lt; 0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Table 2. Effect of administration of aqueous extract of O.g on kidney weight as % of body weight on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days¹</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (normal saline)</td>
<td>0.50 ± 0.01*</td>
<td>0.53 ± 0.01*</td>
<td>0.50 ± 0.01*</td>
<td>0.51 ± 0.01*</td>
<td>0.52 ± 0.01*</td>
<td>0.51 ± 0.01*</td>
</tr>
<tr>
<td>2. Cis (5mg/kg i.p)+2ml water</td>
<td>0.52 ± 0.01*</td>
<td>0.62 ± 0.02*</td>
<td>0.62 ± 0.01*</td>
<td>0.62 ± 0.01*</td>
<td>0.61 ± 0.00*</td>
<td>0.62 ± 0.01*</td>
</tr>
<tr>
<td>3. Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>0.51 ± 0.01*</td>
<td>0.62 ± 0.00*</td>
<td>0.59 ± 0.01*</td>
<td>0.55 ± 0.01*</td>
<td>0.54 ± 0.00*</td>
<td>0.55 ± 0.01*</td>
</tr>
<tr>
<td>4. Cis (5mg/kg i.p)+2ml 10% O.G</td>
<td>0.51 ± 0.01*</td>
<td>0.62 ± 0.01*</td>
<td>0.59 ± 0.01*</td>
<td>0.55 ± 0.01*</td>
<td>0.55 ± 0.00*</td>
<td>0.53 ± 0.01*</td>
</tr>
<tr>
<td>Results of one-way ANOVA F-value; P-value</td>
<td>2.16; p&gt; 0.05</td>
<td>48.47; p&lt; 0.05</td>
<td>44.26; p&lt; 0.05</td>
<td>53.02; p&lt; 0.05</td>
<td>44.85; p&lt; 0.05</td>
<td>51.72; p&lt; 0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Table 3. Effect of administration of aqueous extract of O.g on serum urea (mmol/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Serum Urea (mmol/l)</th>
<th>0days¹</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (normal saline)</td>
<td>6.67 ± 0.06*</td>
<td>5.52 ± 0.01*</td>
<td>5.57 ± 0.15*</td>
<td>6.00 ± 0.10*</td>
<td>5.83 ± 0.06*</td>
<td>5.20 ± 0.10*</td>
</tr>
<tr>
<td>2. Cis (5mg/kg i.p)+2ml water</td>
<td>5.73 ± 0.06*</td>
<td>14.23 ± 0.11*</td>
<td>13.90 ± 0.10*</td>
<td>13.73 ± 0.20*</td>
<td>14.03 ± 0.12*</td>
<td>13.00 ± 0.10*</td>
</tr>
<tr>
<td>3. Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>5.97 ± 0.11*</td>
<td>14.00 ± 0.10*</td>
<td>12.03 ± 0.11*</td>
<td>8.77 ± 0.11*</td>
<td>8.43 ± 0.43*</td>
<td>7.40 ± 0.10*</td>
</tr>
<tr>
<td>4. Cis (5mg/kg i.p)+2ml 10% O.G</td>
<td>5.43 ± 0.08*</td>
<td>13.82 ± 0.18*</td>
<td>11.58 ± 0.11*</td>
<td>9.90 ± 0.10*</td>
<td>8.00 ± 0.09*</td>
<td>7.42 ± 0.06*</td>
</tr>
<tr>
<td>Results of one-way ANOVA F-value; P-value</td>
<td>145.97; p&lt; 0.05</td>
<td>462.08; p&lt; 0.05</td>
<td>1785; p&lt; 0.05</td>
<td>1217; p&lt; 0.05</td>
<td>3245; p&lt; 0.05</td>
<td>1323; p&lt; 0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Research Article
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Indian J. Drugs Dis.
### Table 4. Effect of administration of aqueous extract of O.g on serum creatinine (umol/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days²</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>69.73 ± 0.25</td>
<td>74.30 ± 0.30²</td>
<td>69.87 ± 0.25²</td>
<td>73.13 ± 0.32²</td>
<td>73.07 ± 0.25²</td>
<td>74.33 ± 0.67²</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) + 2ml O.G</td>
<td>72.17 ± 0.42²</td>
<td>159.53 ± 0.85²</td>
<td>155.67 ± 0.49²</td>
<td>150.30 ± 0.80²</td>
<td>147.00 ± 0.26²</td>
<td>149.70 ± 0.46²</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p) + 2ml 5% O.G</td>
<td>68.50 ± 0.50²</td>
<td>163.233 ± 0.38²</td>
<td>128.77 ± 1.40²</td>
<td>121.73 ± 0.91²</td>
<td>110.63 ± 0.25²</td>
<td>99.73 ± 0.35²</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) + 2ml 10% O.G</td>
<td>73.17 ± 0.25²</td>
<td>157.10 ± 0.36²</td>
<td>133.23 ± 1.17²</td>
<td>104.43 ± 1.08²</td>
<td>105.57 ± 1.21²</td>
<td>84.37 ± 0.61²</td>
</tr>
</tbody>
</table>

Results of one-way ANOVA followed by post-hoc LSD and Turkey.

Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05.

1Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 737.65, p<0.05. Treatment effect, p<0.05, F = 13760; time effect, p < 0.05, F = 30580.

### Table 5. Effect of administration of aqueous extract of O.g on serum uric acid (umol/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days²</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>127.90 ± 2.42²</td>
<td>133.63 ± 0.67²</td>
<td>127.00 ± 1.25²</td>
<td>124.53 ± 0.60²</td>
<td>132.43 ± 0.50²</td>
<td>129.63 ± 0.95²</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) + 2ml O.G</td>
<td>133.30 ± 2.75²</td>
<td>208.33 ± 2.43²</td>
<td>246.20 ± 1.35²</td>
<td>240.47 ± 1.35²</td>
<td>236.80 ± 1.05²</td>
<td>219.80 ± 1.10²</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p) + 2ml 5% O.G</td>
<td>135.50 ± 1.91²</td>
<td>206.70 ± 2.20²</td>
<td>246.37 ± 2.18²</td>
<td>218.90 ± 1.73²</td>
<td>192.53 ± 1.16²</td>
<td>183.03 ± 1.51²</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) + 2ml 10% O.G</td>
<td>134.30 ± 2.13²</td>
<td>210.27 ± 1.53²</td>
<td>241.50 ± 1.61²</td>
<td>209.53 ± 0.57²</td>
<td>179.23 ± 0.85²</td>
<td>153.87 ± 0.21²</td>
</tr>
</tbody>
</table>

Results of one-way ANOVA followed by post-hoc LSD and Turkey.

Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05.

1Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 2556, p<0.05. Treatment effect, p<0.05, F = 9938; time effect, p < 0.05, F = 3867.

### Table 6. Effect of administration of aqueous extract of O.g on serum albumin (g/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days²</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>56.50 ± 0.87³</td>
<td>57.70 ± 0.46³</td>
<td>58.57 ± 0.61³</td>
<td>56.90 ± 0.20³</td>
<td>56.93 ± 0.15³</td>
<td>55.47 ± 0.49³</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) + 2ml water</td>
<td>57.03 ± 0.11³</td>
<td>33.43 ± 0.70³</td>
<td>27.63 ± 0.67³</td>
<td>26.27 ± 0.40³</td>
<td>27.57 ± 0.76³</td>
<td>32.23 ± 1.03³</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p) + 2ml 5% O.G</td>
<td>58.67 ± 2.39³</td>
<td>35.57 ± 0.76³</td>
<td>28.80 ± 0.36³</td>
<td>37.27 ± 0.81³</td>
<td>40.10 ± 0.40³</td>
<td>43.70 ± 0.53³</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) + 2ml 10% O.G</td>
<td>59.10 ± 1.00³</td>
<td>33.97 ± 0.31³</td>
<td>30.67 ± 0.49³</td>
<td>38.00 ± 0.10³</td>
<td>45.47 ± 1.12³</td>
<td>48.77 ± 0.31³</td>
</tr>
</tbody>
</table>

Results of one-way ANOVA followed by post-hoc LSD and Turkey.

Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05.

1Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 2773; time effect, p < 0.05, F = 1125.

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**Table 4. Effect of administration of aqueous extract of O.g on serum creatinine (umol/l) on cisplatin induced nephrotoxicity in rats¹**

**Table 5. Effect of administration of aqueous extract of O.g on serum uric acid (umol/l) on cisplatin induced nephrotoxicity in rats¹**

**Table 6. Effect of administration of aqueous extract of O.g on serum albumin (g/l) on cisplatin induced nephrotoxicity in rats¹**
Table 7. Effect of administration of aqueous extract of O.g on urine volume (ml/24hr) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>7.40 ± 0.20</td>
<td>7.60 ± 0.26</td>
<td>8.27 ± 0.15</td>
<td>8.07 ± 0.15</td>
<td>8.63 ± 0.31</td>
<td>8.50 ± 0.53</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) +2ml water</td>
<td>8.63 ± 0.31</td>
<td>39.36 ± 0.86</td>
<td>39.90 ± 0.20</td>
<td>35.60 ± 0.36</td>
<td>34.67 ± 0.32</td>
<td>27.13 ± 0.67</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>7.46 ± 0.13</td>
<td>37.30 ± 0.17</td>
<td>32.70 ± 0.40</td>
<td>22.50 ± 0.36</td>
<td>15.90 ± 0.20</td>
<td>12.17 ± 0.31</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) +2ml 10% O.G</td>
<td>8.07 ± 0.15</td>
<td>37.93 ± 0.15</td>
<td>28.90 ± 0.20</td>
<td>19.90 ± 0.20</td>
<td>13.80 ± 0.46</td>
<td>11.43 ± 0.50</td>
</tr>
<tr>
<td>Results of one-way ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F- value; P- value</td>
<td>45.84; p&lt; 0.05</td>
<td>2360;p&lt; 0.05</td>
<td>4451;p&lt; 0.05</td>
<td>2433;p&lt; 0.05</td>
<td>1935;p&lt; 0.05</td>
<td>739.49; p&lt; 0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Table 8. Effect of administration of aqueous extract of O.g on urinary protein (g/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0 days</th>
<th>3 days</th>
<th>6 days</th>
<th>9 days</th>
<th>12 days</th>
<th>15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>0.38 ± 0.02</td>
<td>0.37 ± 0.01</td>
<td>0.37 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) +2ml water</td>
<td>0.38 ± 0.02</td>
<td>3.57 ± 0.01</td>
<td>3.58 ± 0.01</td>
<td>3.59 ± 0.01</td>
<td>3.55 ± 0.05</td>
<td>3.66 ± 0.03</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>0.37 ± 0.01</td>
<td>3.59 ± 0.01</td>
<td>2.97 ± 0.04</td>
<td>2.17 ± 0.06</td>
<td>1.48 ± 0.08</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) +2ml 10% O.G</td>
<td>0.36 ± 0.01</td>
<td>3.57 ± 0.01</td>
<td>2.85 ± 0.05</td>
<td>2.00 ± 0.05</td>
<td>1.33 ± 0.05</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>Results of one-way ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F- value; P- value</td>
<td>1.48; p&gt; 0.05</td>
<td>48010; p&lt;0.05</td>
<td>3306;p&lt;0.05</td>
<td>1947;p&lt;0.05</td>
<td>1340;p&lt;0.05</td>
<td>21370;p&lt;0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Table 9. Effect of administration of aqueous extract of O.g on urinary creatinine(umol/l) on cisplatin induced nephrotoxicity in rats¹

<table>
<thead>
<tr>
<th>Groups/Treatment</th>
<th>0days</th>
<th>3days</th>
<th>6days</th>
<th>9days</th>
<th>12days</th>
<th>15days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Control (normal saline)</td>
<td>9729.00 ± 37.99</td>
<td>9727.70 ± 54.50</td>
<td>9770.03 ± 54.00</td>
<td>9768.30 ± 41.93</td>
<td>9757.70 ± 42.72</td>
<td>9795.00 ± 4.58</td>
</tr>
<tr>
<td>2.Cis (5mg/kg i.p) +2ml water</td>
<td>9759.30 ± 42.91</td>
<td>2214.30 ± 38.73</td>
<td>2205.30 ± 48.01</td>
<td>2223.30 ± 28.00</td>
<td>2417.30 ± 31.21</td>
<td>2246.70 ± 46.61</td>
</tr>
<tr>
<td>3.Cis (5mg/kg i.p)+2ml 5% O.G</td>
<td>9783.30 ± 50.54</td>
<td>2194.00 ± 39.00</td>
<td>4188.00 ± 43.41</td>
<td>6329.30 ± 24.58</td>
<td>7488.70 ± 25.69</td>
<td>9749.00 ± 26.29</td>
</tr>
<tr>
<td>4.Cis (5mg/kg i.p) +2ml 10% O.G</td>
<td>9748.70 ± 29.26</td>
<td>2213.30 ± 44.47</td>
<td>4335.30 ± 35.66</td>
<td>6422.00 ± 30.41</td>
<td>7644.30 ± 31.63</td>
<td>9690.00 ± 39.95</td>
</tr>
<tr>
<td>Results of one-way ANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F- value; P- value</td>
<td>5.56;p&lt;0.05</td>
<td>52260;p&lt;0.05</td>
<td>32960;p&lt;0.05</td>
<td>877400;p&lt;0.05</td>
<td>387600;p&lt;0.05</td>
<td>152800;p&lt;0.05</td>
</tr>
</tbody>
</table>

¹Data are Mean ± SD (n = 3). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD and Turkey).

Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 301800, p<0.05. Treatment effect, p<0.05, F = 9330.

Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 9330.
Moreover, a significant (P≤0.05) increase in kidney weight as % of body weight was noticed in cisplatin treated rats after 15 days when compared with the normal (control) (Table 2). Also, the administration of cisplatin along with various concentrations (5% and 10%) of the aqueous extract (O.G.) decreased kidney weight as % of body weight significantly (P≤0.05) in all groups receiving treatment with the extracts after 15 days when compared with the cisplatin treated group (Table 2).

The decrease in kidney weight as % of the body weight by extracts though not statistically significant (P≥0.05) was dose dependent. Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on kidney weight as % of body weight (P≤0.05) (Table 2).

Intraperitoneal administration of cisplatin (5mg/kg i.p.) caused abnormal renal function in all rats. Serum urea, creatinine and uric acid increased (P≤0.05) in the group treated with cisplatin only, after 6 days when compared with the normal (control). There were, however, slight decreases (P≥0.05) on the 9th and 15th day (Table 3, 4 and 5).

The decrease in serum urea, creatinine and uric acid of rats exposed to cisplatin and the various concentrations (5% and 10%) of aqueous extract of Ocimun gratissimum in groups 3 and 4 respectively increased significantly on the 3rd day but decreased on the 6th, 9th, 12th and 15th day when compared to the cisplatin treated group. (P≤0.05) (Table 3, 4 and 5).

Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on serum albumin (P≤0.05) (Table 6).

Cisplatin treatment also produced a significant increase (P≤0.05) in urine volume and urinary protein towards the 6th day but decreased (P≥0.05) on the 9th, 12th and 15th day when compared to the normal (Tables 7-8). There were however slight increases (P≥0.05) on the 15th day for urinary protein.

The decrease in urine volume and urinary protein by the extract though not statistically significant (P≥0.05) was dose dependent. The effect of time of administration of aqueous extract of O. gratissimum on urine volume and urinary protein were statistically significant (P≤0.05).

Two way analysis of variance indicated that cisplatin and the extract showed significant interaction between time and doses on urinary creatinine (P≤0.05) (Table 6).

A marked (P≤0.05) decrease in the levels of urinary creatinine was noticed in cisplatin treated rats after 15 days when compared with the normal (control) (Table 9). Also, the administration of cisplatin along with various concentrations of the aqueous extracts O.G. significantly increased (P≤0.05) urinary creatinine in all groups receiving treatment with the extracts after 15 days when compared with the cisplatin treated group. (Table 9).

The increase in urinary creatinine by extracts though not statistically significant (P≥0.05) was dose dependent. The effect of time of administration of aqueous extract of O. gratissimum on urinary creatinine was statistically significant (P≤0.05). Two way analysis of variance indicated that cisplatin and the extracts showed significant interaction between time and doses on urinary creatinine (P≤0.05) (Table 9).
The kidney of rats in group 1 showed a normal architecture, normal organization of tubular epithelial cells and glomeruli cells (Plate 1).

In cisplatin treated kidney, drastic alterations were observed. Histopathological examination showed severe degeneration in tubular cells, congestion of glomeruli and infiltration of interstitium by inflammatory cells (Plate 2).

5% O.G + Cisplatin and 10 % O.G + Cisplatin treated kidney which are the test groups (3 and 4) generally showed defects observed in the cisplatin treated rats. There was significant improvement when compared with cisplatin treated kidney (Plate 3 and 4).

**Discussion**

In recent years, due to various reasons the number of persons suffering from renal problems is increasing, the exposure to certain drugs such as cisplatin, gentamicin and paracetamol is one of the causes of renal toxicity. Till date there are no drugs available which could effectively prevent the incidence/development or cure the renal damage caused by various agents.

Many compounds (synthetic and natural) are screened for nephroprotective activity against cisplatin induced nephrotoxicity. In these studies treatment with plant extracts is quite encouraging (Mohan et al., 2006; Sreedevi et al., 2009; Mohamed et al., 2010).
Acute renal failure (ARF) is characterized by a rapid decline in glomerular filtration rate (GFR) over hours to days, and the retentions of nitrogenous waste products. The mortality rate of patients with ARF has remained 25-70% despite the use of various pharmacologic agents (Dong et al., 2009).

Therefore, a new therapeutic agent is required to promote renal function recovery. Cisplatin, a heavy metal complex, is an effective chemotherapeutic agent for a wide variety of tumors (Park et al., 2009). Nevertheless, it has several toxicities and side effects including hepatotoxicity (Mansour et al., 2006; Pratibha et al., 2006) and nephrotoxicity (Park et al., 2009). Lipid peroxidation (LPO) is crucial in the pathogenesis of cisplatin-induced organ injury (Weji et al., 1997; Autunes, 2000; Autunes et al., 2001; Mora et al., 2003). Cisplatin causes the generation of oxygen free radicals, such as hydrogen peroxide, and hydroxyl radical, which abstract a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation (Kadikoylu et al., 2004). In addition, nitric oxide, with high spontaneous chemical reactivities, can react with superoxide to generate peroxynitrite (Aoyagi et al., 1999), which has been suggested as a main source of hydroxyl radical in many pathological conditions (Daloz et al., 1992; Obata, 2002). Lipid peroxidation is important in the pathogenesis of cisplatin-induced hepatic and renal injuries (Baliga et al., 1999).

The cytotoxicity caused by cisplatin is considered to be due to several factors viz., the peroxidation of cell membranes, mitochondrial dysfunction, inhibition of protein synthesis, and DNA damage (Kharbanda et al., 1995; Leibbrandt et al., 1995; Brady et al., 1999). Many antioxidative agents have been analyzed in experimental and clinical studies searching for an agent to reduce or prevent cisplatin-induced nephrotoxicity (Autunes et al., 2000; Davies et al., 2001; Dillioglugil et al., 2005).

Much attention has been given to the possible role of dietary antioxidants in protecting liver against cisplatin-induced toxicity (Behling et al., 2006). Phytochemicals, including flavonoids, are naturally occurring antioxidants that possess various pharmacological actions and therapeutic applications (Hasanloo et al., 2005; Karimi et al., 2005). So, due to their phenolic structures they inhibit free radical-mediated processes (Singh et al., 2005). Many antioxidant compounds have been studied as chemoprotective agents such as, curcumin, selenium and other dietary components that scavenge free radicals formed by exposure to cisplatin (Antunes et al., 2001; Silva et al., 2001).

Most studies reported previously, were designed to administer drugs before or at the same time of renal insult. However, most therapeutic agents are usually administered after the expression of clinical diseases. Therefore it was hypothesized that Ocimum gratissimum (O.G.) might affect the course of tubular repair after the onset of cisplatin-induced nephrotoxicity and thus, accelerate recovery in the rats.

Chemotherapeutic levels of cisplatin known to induce renal and hepatic injury in rats is thought to be a single dose of 5 mg/kg body weight which peaks in about 3 – 5 days (Stein et al., 1978; Singh, 1989; Okoko and Orambo, 2008) thus the choice of a single dose of 5 mg/kg body weight, and the three days exposure before the administration of the aqueous extracts of O. gratissimum for the present study.

Nephrotoxicity in this study was evaluated by body weight, kidney weight to body weight ratio and renal markers (urea, creatinine, uric acid, albumin and 24 hr urinary factors which include urine volume, urinary creatinine and protein). Recent studies have been focused on the ways for protection against cisplatin-induced nephrotoxicity (Mansour et al., 2006; Nagizadeh et al., 2008 Ibrahim et al., 2010; Sreedevi et al., 2010; Mohamed et al., 2010; Noori & Mahboob, 2010). However, the literature is devoid of any reports on O. gratissimum against cisplatin nephrotoxicity.
In the present investigation a single dose of cisplatin (5 mg kg\(^{-1}\)), in male albino rats resulted in significant body weight reduction and decreased food intake. (Table 1) In accordance with present results, Chirino et al. (2004) suggested that after 3 days i.p. administration of a single dose of cisplatin to male Wistar rats (7.5 mg kg\(^{-1}\)) significantly depressed their body weight. Confirming our point of view, Shimeda et al. (2005) and Norrgren et al. (2006), stated that cisplatin has been shown to decrease total body weight in male Sprague Dawley rats and Wistar rats, respectively. Mora et al. (2003) suggested that cisplatin induced weights loss might be due to gastrointestinal toxicity and by reduced ingestion of food.

Post-treatment of aqueous extract of Ocimum gratissimum (O.G.) three days after cisplatin injection remarkably ameliorated the reduction in body weight induced by cisplatin (Table 1). Cisplatin induced kidney damage was characterized by a significant increase (P≤0.05) in the kidney weight to body weight ratio (Table 2). This result was consistent with that of Mansour et al. (2002), who indicated that a single dose of cisplatin (7.5mg/kg i.p.) in rats resulted in kidney weight loss manifested by significant depression as a percentage of the total body weight. It could be elucidated that alterations of kidney weight to body weight ratio in cisplatin intoxicated rats could be due to tissue damage and reduction in their functions as reported by Lee et al. (2007) and Park et al. (2009).

Post-treatment of aqueous extract of O.gratissimum returned the kidney weight to body weight ratio close to normal after 15 days. Post-treatment of aqueous extract of O.gratissimum resulted in higher significant elevation of these values compared to cisplatin treated groups (Table 2).

As predicted, administration of a single dose of cisplatin (5mg/kg) induced nephrotoxicity, manifested biochemically by a significant elevation in serum urea, creatinine, uric acid and a severe decrease in serum albumin. Moreover marked increases in urine volume, urinary protein and decrease in urinary creatinine were observed (Tables 3-9).

Oral administration of aqueous extract of O.gratissimum (5% and 10%) after cisplatin administration caused a decline in nephrotoxicity after 15 days for rats treated with cisplatin. This was evidenced by marked decrease in serum urea, creatinine, uric acid and increase in serum albumin concentration of those treated with O. gratissimum extract relative to the group treated with cisplatin alone (Tables 3-6).

Marked increases were observed in urine volume, urinary protein and decrease in urinary creatinine in rats treated with cisplatin alone. There were however significant (P≤0.05) reduction in urine volume and urinary protein and a marked increase (P≤0.05) in urinary creatinine and creatinine clearance in rats treated with cisplatin and 5 and 10% of aqueous extracts of O.gratissimum. (Tables 7-9).

This marked decrease in serum urea, creatinine, uric acid , urine volume and urinary protein and increased urinary creatinine with administration of aqueous leaf extracts of O.gratissimum in cisplatin induced nephrotoxicity was in agreement with studies by other researchers (Mansour et al., 2006; Nagizadeh et al.,2008; Ibrahim et al.,2010; Sreedevi et al.,2010; Mohamed et al.,2010; Noori and Mahboob, 2010).

Noori and Mahboob (2010) reported that administration of cisplatin to rats caused a reduction in glomerular filtration rate, which correlated with increased creatinine and urea in plasma. This is in agreement with the present study which showed that administration of cisplatin to rats caused a reduction in glomerular filtration rate, which correlated with alteration in the renal function as indicated by the different values of renal markers (Tables 3-9). The alterations in glomerular function in cisplatin treated rats may also be secondary to ROS (reactive oxygen species) (Somani et al., 2000) which induce mesangial cells contraction, altering the filtration surface area and modifying the
ultrafiltration coefficient factors that decrease the glomerular filtration rate (Aydogan et al., 2008).

Polyuria uniformly accompanies cisplatin administration and occurs in two distinct phases. Phase one occurs within the first two days after administration but resolves spontaneously. It is characterised by decreased urine osmolality but stable glomerular filtration rate (GFR). Phase two starts between three to four days after administration and is characterized by decreased GFR, urea cycling effect which causes derangement in ionic metabolism of sodium, potassium, magnesium and calcium (Yao et al., 1988).

The polyuria observed after injection with cisplatin might be related to a significant decrease of collecting duct water channels (aquaporins 2 and 3), as well as to the increase in renal sodium excretion observed in cisplatin-injected rats (Daugaard et al., 1988). These biochemical findings were further confirmed by evidences of microscopic examinations. The kidney of rats in group 1 showed a normal architecture, normal organization of tubular epithelial cells and glomeruli cells (Plate 1).

In cisplatin treated kidney, drastic alterations were observed. Histopathological examination showed severe degeneration in tubular cells, congestion of glomeruli and infiltration of interstitium by inflammatory cells (Plate 2).

5% O.G + Cisplatin and 10% O.G + Cisplatin treated kidney which are the test groups (3 and 4) generally showed defects observed in the cisplatin treated rats. There was significant improvement when compared with cisplatin treated kidney (Plate 3 & 4).

Phytochemical screening of the leaf extract of Ocimum gratissimum (O.G) had shown the plant to contain alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides (Holets et al., 2003; Akinwumi et al., 2005; Akinmoladun et al., 2007). Flavonoids are reported to exhibit antioxidant activity (Ramanathan et al., 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988). The aqueous extract of O.gratissimum may have exhibited anti-nephrotoxic activity due to its possible antioxidant content attributable to flavonoids. Interestingly, saponins especially terpene glycosides are reported to enhance natural resistance and recuperative powers of the body (Singh et al., 1991) and O. gratissimum has been shown to be a rich source of this compound (Prabhu et al., 2009; Obianime et al., 2011).

However, no study has focused on the antinephrotoxic effect of Ocimum gratissimum on cisplatin-induced toxicity. In conclusion, the results of this study indicate that aqueous leaf extracts of Ocimum gratissimum affect the course of tubular repair after the onset of cisplatin-induced nephrotoxicity in rats with accelerated recovery.

References


34. Mansour HH, Hafez HF and Fahmy NM (2006) Silymarin modulates Cisplatin 10 mg kg⁻¹ platin
induced oxidative stress and hepatotoxicity in rats. 


