Hepatoprotective activity of *Oxalis corniculata* L. ethanolic extract against paracetamol induced hepatotoxicity in Wistar rats and its *in vitro* antioxidant effects

G Sreejith, M Jayasree¹, P G Latha*, S R Suja, S Shyamal, V J Shine, G I Anuja, S Sini,
P Shikha, N M Krishnakumar, V Vilash, S Shoumya & S Rajasekharan
Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode,
Thiruvananthapuram 695 562, India
¹ N S S College, Nilamel, Kollam 691 535, India

Received 26 November 2012; revised 10 October 2013

*Oxalis corniculata* is well known for its medicinal properties like anti-inflammatory, digestive, diuretic, antibacterial, antiseptic etc. The present study focuses on the ability of *O. corniculata* to alleviate liver damage caused by over dose of paracetamol. Antioxidant activity of *O. corniculata* was evaluated using the free radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radicals, total antioxidant capacity by phosphomolybdenum method and total phenolic content was also evaluated. The ethanolic extract of whole plant of *O. corniculata* (OC, 500 µg/mL, po) significantly reduced 1, 1-diphenyl-2-picrylhydrazyl radicals. This dose also caused significant reduction (62.67%) in malondialdehyde levels of murine hepatic tissues. The antioxidant capacity of OC was comparable to that of standard ascorbic acid and showed 53.5 µg of phenol/mg OC. Rats pre-treated with OC for 4 days showed significant reduction in the serum enzymes such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, serum bilirubin and showed almost normal histological liver architecture of the treated groups compared to paracetamol induced hepatic damage group, indicating its hepatoprotective and antioxidant potential.

**Keywords:** Antioxidant, Hepatoprotective, Lipid peroxidation, Malondialdehyde, *Oxalis corniculata*

Liver diseases are serious ailments, classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals¹. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India².

*Oxalis corniculata* L. is a small procumbent herb commonly known as ‘Indian Sorrel’ in English, ‘Tinpatiya’ in Hindi and ‘Puliyarila’ in Malayalam. *O. corniculata* has been used by the tribal people in Koraput district of Orissa as a folk medicine to treat headache, cure skin diseases and in raw form is good for digestion³,⁴. This plant is well known for its medicinal values as a remover of vata and kapha in Ayurveda. In Indian traditional medicine, a table spoon of fresh leaf juice mixed with buttermilk is taken once daily in the treatment of jaundice, it is used as anti inflammatory, digestive, diuretic, antibacterial, antiseptic and for cardiopathy and hepatopathy. It is also known to cure dysentery, diarrhoea, piles and skin diseases⁵,⁶. *O. corniculata* is the main ingredient of the Unani drug, “Changeri”; the drug is considered to be cooling, appetizing, and useful in fever, biliousness and dysentery⁷. *O. corniculata* has also showed marked antibacterial activity against *E. Coli*⁸. These information prompted to study the ability of *O. corniculata* to alleviate the damage caused by over dose of paracetamol and also its antioxidant potential.

**Materials and Methods**

*Chemicals and instruments—*All chemicals and solvents used in the study were of analytical grade. Paracetamol (Acetaminophen, APAP), ascorbic acid, gallic acid, thiobarbituric acid (TBA), sodium dodecyl
Plant material and preparation of the extract—The whole plant of *O. corniculata* (OC) was collected from various parts of Thiruvananthapuram district, JNTBGRI, Palode (8° 45’ & 8° 47’ North Latitude and 77° 01’ & 77° 04’ East Longitude), Ponmudy (8° 75’ & 8° 76’ North Latitude and 77° 10’ & 77° 11’ East Longitude) and from Poojappura (8° 48’ North Latitude and 76° 97 East Longitude) and were authenticated by Dr. Mathew Dan, plant taxonomist of the Institute. A voucher specimen has been deposited at the Institute Herbarium (TBGT- 57056, of the Institute. Rotary evaporator was from Superfit, India and UV visible spectrophotometer from Remi, India.

**Animals**—Wistar albino male rats, (150–200 g) and male Swiss albino mice, (20–30 g), were obtained from Jawaharlal Nehru Tropical Botanic Garden and Research Institute’s Animal House. They were maintained under standard laboratory conditions (24-28 °C, 60-70% RH and 12:12 h L:D cycles) and fed commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All experiments were carried out according to NIH guidelines, after getting the approval of the Institute’s Animal Ethics Committee. (No B-7/1/2008/123).

**Acute toxicity studies**—Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development (OECD) guidelines. Briefly, four groups of 12 mice were administered 250, 500, 1000 and 2000 mg/kg of the OC extract, po. They were observed continuously for 1 h for any gross changes, symptoms of toxicity and mortality if any and intermittently for the next 6 h and then again, 24 h after dosing with OC extract.

**Paracetamol-induced hepatotoxicity**—Rats were divided into following six groups of 6 each. Group 1 (normal control group) and Group 2 (toxin control group) received 0.5% Tween-80 (2 mL, po), for 4 days. Animals of Groups 3, 4 and 5 were treated with OC (100, 250 and 500 mg/kg, po) for 4 days and animals of Group 6 were given silymarin, the positive control used in the study (100 mg/kg, po) for 4 days. Paracetamol (2.5 g/kg in 0.5% Tween-80, 1.5 mL, po) was administered to Groups 2-6 on the third day 1 h after their respective treatment.

**Biochemical and histopathological studies**—After 48 h paracetamol intoxication, the animals were sacrificed by mild ether anaesthesia. Blood samples were collected in glass test tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation at 3000 g for 20 min and used for evaluating the biochemical parameters and liver tissue slices were collected for histopathological studies.

Biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SAPK) and serum bilirubin were estimated by standard methods. Anti-lipid peroxidation studies—The anti-lipid peroxidant effect of OC was studied *in vitro*, following methods stated elsewhere. Degree of lipid peroxidation in the liver tissue was determined in terms of nmoles of thiobarbituric acid reactive substances (TBARS) produced/mg protein. The absorbance of the samples was measured at 532 nm against a reagent blank and butylated hydroxytoluene (BHT) was used as positive control.

**DPPH radical scavenging activity**—The DPPH radical scavenging activity of OC was measured by the modified method. Briefly to 1 mL of methanolic solution of 0.2 mM DPPH, 0.2 mL of OC dissolved in methanol was added at varying concentrations (100, 200, 300, 500 and 600 µg/mL). The reaction mixture was mixed well and incubated for 20 min at 28°C.
under dark condition. The control contained all reagents except the extract fraction while methanol was used as blank and butylated hydroxyl toluene (BHT) was used as positive control. The DPPH radical scavenging activity was determined by measuring absorbance at 517 nm using a spectrophotometer. The percentage radical scavenging activities of the samples were calculated using the formula \[\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100\].

**Total antioxidant capacity**—The total antioxidant capacity of OC was determined by the standard method and the absorbance was measured at 695 nm against blank. The blank contained the reagent solution without the sample. The total antioxidant activity was expressed as the absorbance value at 695 nm. The higher absorbance value indicates the greater antioxidant activity.

**Determination of total phenolics**—Total phenolic content of OC was determined spectrophotometrically by the standard method and the absorbance was measured at 750 nm. The total phenolic content was determined using the standard gallic acid calibration curve. The estimation was carried out in triplicate. The results were mean±SD expressed as µg of gallic acid equivalent/ mg of extract.

**Statistical analysis**—The data were expressed as mean±SD. The significance of differences among the group was assessed using one way analysis of variance (ANOVA). \(P \leq 0.05\) were considered as significance.

**Results and Discussion**

**Toxicity studies**—In the toxicity study, no mortality occurred within 24 h with the 4 doses of OC tested. The \(L_{D50}\) was therefore greater than 2000 mg/kg, po in mice (unpublished data). The results of the acute toxicity study indicate that OC is fairly nontoxic, up to 2000 mg/kg, po.

**Paracetamol-induced hepatotoxicity**—Paracetamol is a common antipyretic agent which is safe in normal therapeutic dose but can produce fatal hepatic necrosis in man, rats and mice at higher doses. Testing for a potential hepatoprotective agent by paracetamol induced hepatotoxicity has been used as a by several investigators. This is due to the fact that a part of paracetamol is activated by hepatic cytochrome P-450 to a toxic metabolite N-acetyl-p-benzoquinoneimine which causes liver injury by disrupting the transport functions of the hepatocytes resulting in leakage of plasma membrane thereby causing an increase in the serum enzymes like SGOT, SGPT, SAKP and bilirubin. Pretreatment of rats with OC (100 mg/kg) for 4 days prior to paracetamol administration resulted in significant protection from paracetamol induced elevated levels of enzymes indicating its ability to reduce/ reverse the damage (Table 1).

The hepatoprotective effect of OC was further confirmed by histopathological observations. The liver of paracetamol treated rats showed significant damage with coarsely granulated cytoplasm and prominent Kupffer cells. Anucleate hepatocytes were also observed. The rats treated with OC and silymarin, the positive control used in the study showed more or less normal architecture of liver, almost comparable to the normal control group (Fig. 1a-d).

**Anti-lipid peroxidation studies**—Numerous pathological events such as the inflammation process and ageing phenomenon are associated with the generation of reactive oxygen species (ROS) and the induction of lipid peroxidation. The process of lipid peroxidation increases during inflammatory conditions and treatment with plant extract is found to inhibit lipid peroxidation process. \(\text{FeCl}_2\)-ascorbic acid mixture is known to cause lipid peroxidation in rat liver in vitro. In the present study, \(\text{FeCl}_2\)-ascorbic acid treated rat liver homogenate showed a significant increase of

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SAKP (IU/L)</th>
<th>SB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>228.06 ± 1.8</td>
<td>132.61 ± 0.4</td>
<td>125.95 ± 1.2</td>
<td>0.148 ± 0.005</td>
</tr>
<tr>
<td>Paracetamol control</td>
<td>344.94 ± 2.6</td>
<td>150.41 ± 1.5</td>
<td>238.06 ± 2.13</td>
<td>0.631 ± 0.004</td>
</tr>
<tr>
<td>Paracetamol + OC (100 mg/kg)</td>
<td>233.28 ± 1.5*</td>
<td>135.93 ± 1.0*</td>
<td>132.62 ± 1.7*</td>
<td>0.160 ± 0.034*</td>
</tr>
<tr>
<td>Paracetamol + OC (250 mg/kg)</td>
<td>235.89 ± 2.3</td>
<td>137.68 ± 1.4</td>
<td>134.03 ± 1.5</td>
<td>0.198 ± 0.016</td>
</tr>
<tr>
<td>Paracetamol + OC (500 mg/kg)</td>
<td>237.68 ± 2.0</td>
<td>139.42 ± 0.8</td>
<td>143.21 ± 2.5</td>
<td>0.216 ± 0.024</td>
</tr>
<tr>
<td>Paracetamol + Silymarin (100 mg/kg)</td>
<td>230.89 ± 2.7</td>
<td>133.35 ± 1.0*</td>
<td>129.9 ± 1.9*</td>
<td>0.154 ± 0.009*</td>
</tr>
</tbody>
</table>

\(P \leq 0.05\) compared to paracetamol control.
MDA when compared to normal control without FeCl$_2$-ascorbic acid. Treatment of FeCl$_2$-ascorbic acid stimulated liver homogenate with 500 $\mu$g/mL dose of OC showed a decrease in MDA levels by 62.67 %. Butylated hydroxyl toluene (BHT), the positive control used in this study showed 73.33% inhibition of MDA at 50 $\mu$g/mL (Table 2).

**DPPH free radical scavenging activity**—DPPH is a kind of stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity$^{18}$. In this assay the antioxidants in the extract react with DPPH radicals which exists naturally in violet colour, to turn in to yellow, the degree of discolouration indicates the radical scavenging activity$^{32}$. The maximum percentage scavenging activity of OC was 74.20 at 500 $\mu$g/mL.

<table>
<thead>
<tr>
<th>Group</th>
<th>OC (µg/mL)</th>
<th>MDA (n mole/mg protein)</th>
<th>Inhibition of MDA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>-</td>
<td>0.042 ± 0.002</td>
<td>-</td>
</tr>
<tr>
<td>FeCl$_2$-AA control</td>
<td>-</td>
<td>0.15 ± 0.004</td>
<td>-</td>
</tr>
<tr>
<td>FeCl$_2$-AA + OC</td>
<td>50</td>
<td>0.117 ± 0.005</td>
<td>22 ± 1.5</td>
</tr>
<tr>
<td>FeCl$_2$-AA + OC</td>
<td>100</td>
<td>0.095 ± 0.003</td>
<td>36.67 ± 2.9</td>
</tr>
<tr>
<td>FeCl$_2$-AA + OC</td>
<td>250</td>
<td>0.078 ± 0.005</td>
<td>48.00 ± 2.9</td>
</tr>
<tr>
<td>FeCl$_2$-AA + OC</td>
<td>500</td>
<td>0.056 ± 0.001</td>
<td>62.67 ± 1.4$^*$</td>
</tr>
<tr>
<td>FeCl$_2$-AA + BHT</td>
<td>50</td>
<td>0.040 ± 0.002</td>
<td>73.33 ± 1.7$^*$</td>
</tr>
</tbody>
</table>

$^*$P ≤ 0.05, compared to FeCl$_2$ - AA (negative) control
Table 3—Effect of ethanolic extract of O. corniculata whole plant (OC) on DPPH radical scavenging activity

[Values are mean ± SD of 3 replicates]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>OC 50</td>
<td>16.152 ± 0.37</td>
</tr>
<tr>
<td>100</td>
<td>26.966 ± 1.64</td>
</tr>
<tr>
<td>200</td>
<td>49.391 ± 0.37</td>
</tr>
<tr>
<td>300</td>
<td>62.594 ± 0.80</td>
</tr>
<tr>
<td>500</td>
<td>74.204 ± 0.70</td>
</tr>
<tr>
<td>600</td>
<td>72.987 ± 0.05</td>
</tr>
<tr>
<td>BHT (50)</td>
<td>79.213 ± 0.02</td>
</tr>
</tbody>
</table>

BHT, the positive control used in this study showed 79% free radical scavenging activity at 50 µg/mL. The free radical scavenging was observed to be concentration dependent and at lower doses failed to evoke significant response (Table 3).

The total antioxidant capacity—It was determined spectrophotometrically through phosphomolybdenum method, which is dependent on the capacity of OC to reduce MO (VI) to MO (V) resulting in the formation of green phosphomolybdenum (V) with maximum absorption at 695 nm. The antioxidant capacity of OC was found to increase in a concentration dependent manner and showed highest capacity of 1.8 at 100 µg/mL which was comparable to that of ascorbic acid with 1.9 at 100 µg/mL indicating it is good antioxidant agent. Antioxidant capacity of ascorbic acid has been used as a reference standard by which plant extracts with potential antioxidant activity are compared.

Antioxidant capacity of a plant is closely correlated with their phenolic content. In the present study OC showed the presence of about 53.5 µg of gallic acid equivalents of phenol/ mg of OC. Previous phytochemical studies have shown the presence of certain flavonoids like vitexin, isovitexin and vitexin-2-O-β-D-glucopyranosides may be playing a major part in quenching reactive oxygen species (ROS) as evident from the above antioxidant activity studies, thereby improving membrane stability and reducing tissue damage. Further studies are required to understand the exact mechanism of action of OC.

Acknowledgement
Thanks are due to Sri. K P Pradeepkumar for photographic assistance, Sri. S Radhakrishna Pillai for technical assistance and the University Grants Commission (UGC), Government of India for financial assistance.

References
4. Das P K & Misra M K, Some medicinal plants used by the tribals of Deomali and adjacent areas of Koraput district, Orissa, Indian J Forestry, 10 (1987) 301.