International Journal of Pharmacology

ISSN 1811-7775
Evaluation of Antioxidant, Antinociceptive Activities of *Oxalis corniculata* in Diabetic Neuropathy Rats

V. Sampath Kumar, 2V. Venumadhav, 3K. Jagadeeshwar, 1B. Bhaskar and 1Mangala Lakkar

1National Institute of Pharmaceutical Education and Research, Guwahati, Assam India
2Vishnu Institute of Pharmaceutical Education and Research, Narsapur, A.P. India
3St. Mary’s College of Pharmacy, Secunderabad, A.P. India

**Abstract:** Diabetic neuropathy is damage to nerves in the body that occurs due to high blood sugar levels from diabetes. In this study we tried to evaluate the antioxidant, antinociceptive activities of ethanolic extract of *Oxalis corniculata* in experimentally induced diabetic rats. At the end of the 5th week, Diabetic rats were shown significant reduction in tail flick latency by 49% in hot water tail immersion test and decreased paw withdrawal response by 47% in hot plate test. Treatment with extract (400 mg kg⁻¹) was showed a significant increase in reduced glutathione (GSH) levels. After 4 weeks treatment with extract was showed significantly increase in Superoxide dismutase (SOD) activity, treatment with extract 200 mg kg⁻¹ and 400 mg kg⁻¹ significantly decrease in catalase activity, MDA and significant reduction as dose dependant manner in plasma nitrite levels in diabetic rats.

**Key words:** Diabetic neuropathy, hyperalgesia, *Oxalis corniculata*, peroxidation, enzymatic activity

**INTRODUCTION**

Diabetes is a chronic disease which made more than 220 million people diabetic all over the world and 3.4 million people died every year (WHO). Hyperglycemia is an important causative factor to the diabetic complications like retinopathy, nephropathy and neuropathy. Neuropathic pain is a common symptom of diabetic neuropathy which can be evaluated by mechanical and thermal hyperalgesia (Dyck et al., 2000, Negi et al., 2010). Etiology of diabetic neuropathy is partly unclear and characterized by several pathways that includes increased activation of polyol pathway, formation of advanced glycation end products and protein kinase C activation (Nakamura et al., 1999; Taliyan and Sharma, 2010), increased activation of desmine pathway, oxidative-nitrosative stress, poly (ADP-ribose) polymerase activation (Olga et al., 2006), mitogen-activated protein kinases (MAPKs) (Purves et al., 2001) and nerve growth factor deficit (Obrosova et al., 2001) are lead to diabetic neuropathy.

Increased production of reactive oxygen species and decreased antioxidant defense leads to the oxidative stress. Oxidative stress can cause tissue injury or even cell death which can occur by necrosis and apoptosis (Adly, 2010). In hyperglycemia, superoxide is the first free radical produced in the mitochondria or cell (Brownlee, 2001). Other reactive species includes hydroxyl, hydrogen peroxyde and peroxinitrite shows detrimental effects in the tissue of diabetic rats (Cameron et al., 2001). This includes accumulation of conjugated dienes and decreased levels of antioxidant enzymes contribute to increased evidence of oxidative stress. This is an important factor in the pathogenesis of diabetic neuropathy.

*Oxalis corniculata* L. (*Oxalidaceae*) is a perennial herb and distributed all over the India, especially in North-East region. The whole plant is a good source for vitamin C and leaves contain C-glycosyl flavones i.e., isocorixin, isovitexin and sertisin (Mizokami et al., 2008). Several experimental studies were shown, the plant having wound healing (Taranalli et al., 2004), antibacterial (Rahman et al., 2010; Satish et al., 2008), antifungal (Iqbal et al., 2001), Anti-implantation, abortifacient (Sharangouda and Patil, 2007), anti-epileptic (Kumar and Rajkapoorn, 2010), antitumor and antioxidant activity (Kathiriya et al., 2010; Alam et al., 2011). It also reported to exhibits hypoglyceanic, antihypertensive, antipsychotic, CNS-stimulant, chronotropic and inotropic effect (Achola et al., 1995; Alam et al., 2011). Aqueous extract of *Oxalis corniculata* protect cardiac injury marker enzymes and maintain the activity of lipogenic enzyme, glucose-6-phosphate dehydrogenase (Upagamalwar et al., 2011). Traditionally it is used to relief the pain and in rheumatism (Libman et al., 2006) and the whole plants along with ginger is made a paste and applied for dysentery (Siddique et al., 2006). Most of the flavonoids and polyphenols are useful in Oxidative-nitrosative stress...
involved in chronic diseases. The present study evaluated ethanolic extract of *O. corniculata* on nociception and oxidative stress in streptozotocin (STZ)-induced diabetic neuropathy in rats.

MATERIALS AND METHODS

**Plant material:** Whole plant of *O. corniculata* was collected from Narakasur hill top, around Guwahati Medical College and authenticated in Department of Botany, Guwahati University. The plant sample has been kept in voucher specimen (No. 01577) at NIPER, Guwahati-India.

**Extraction:** Fresh whole plant of *O. corniculata* was shed dried at room temperature and powdered. The powder was soaked in 50% ethanol for 3 days with intermittent shaking. The extract was filtered and the solvent was completely removed at 50°C under reduced pressure. The yield of extract was 18% (w/w) in terms of starting material. The dried extract was stored in refrigerator at 2-8°C for further use. The extract was found to contain flavonoids, carbohydrates, alkaloids, steroids and tannins.

**Reagents:** All reagent grade quality chemicals and biochemical assay kits were purchased from Sigma, India. Thiobarbituric acid (TBA), DTNB (5, 5'-dithio, bis 2-nitrobenzoic acid) were purchased from Himedia laboratories, India.

**Animals:** Healthy male Sprague-Dawley rats (200-250 g, 9-12 weeks age) were obtained from the Central Animal Facility (CAF), NIPER-Guwahati. Animals were fed on standard rat diet and water *ad libitum*. They were housed in plastic cages at a controlled temperature of 24±1°C and humidity 55±5%, with 12 h light and 12 h dark cycle. All animals were acclimatized for minimum period of 1 week prior to the beginning of study. All the experimental protocols were approved by Institutional Animal Ethics committee and performed according to the Indian National Science Academy Guidelines for the care and use of animals in scientific research.

**Induction of diabetes and Experimental design:** Diabetes was induced by a single dose streptozotocin (50 mg kg⁻¹ b.wt., i.p.) in citrate buffer (pH 4.5, 0.1 M). Blood samples for glucose measurement were taken from tail vein 48 h after the STZ injection and the day before the animals were killed. The rats with blood glucose levels more than 250 mg dL⁻¹ were considered as diabetic and were used for further study. The duration of experiment was 8 weeks.

**Group 1:** Control group-vehicle treated
**Group 2:** Diabetic control-vehicle treated
**Group 3:** Ethanolic extract of *Oxalis corniculata* (EEOC) 200 mg kg⁻¹ b.wt. orally
**Group 4:** Ethanolic extract of *Oxalis corniculata* (EEOC) 400 mg kg⁻¹ b.wt. orally

The treatment was started after 4 weeks of STZ injection and continued up to 8th week. Thermal hyperalgesia was assessed after 5th, 6th, 7th and 8th week of STZ injection. At the end of the study, the animals were anaesthetized with ketamine (50 mg kg⁻¹ b.wt. i.p) and then killed. The left and right sciatic nerves were rapidly excised, cleaned and homogenized and all the biochemical parameters were measured 24 h after the last dose.

**Assessment of thermal hyperalgesia:** Hyperalgesia was assessed by tail immersion (warm water) test and hot plate test (Kannan et al., 1996; Ramabadran et al., 1989).

**Tail-immersion (warm water) test:** The animals were trained for 3 days prior to test. Rat tail was immersed in hot (52±0.5°C) water and the tail flick response latency (withdrawal response) or any signs of struggle were observed as the end point response. Cut off time was kept at 12 sec. shortening of tail withdrawal time indicates hyperalgesia. The test was repeated 3 times within 30 min.

**Hot-plate test:** Hyperalgesic response on the hot plate is considered to result from a combination of central and peripheral mechanism (Kannan et al., 1996). Animals were individually placed into a glass chamber (Eddy’s Hot-plate) with the temperature adjusted to 55±1°C (Jothimaniyvanan et al., 2010). The latency of first reaction (licking, moving the paws, little leaps or a jump to escape the heat) was recorded with cut off time was kept at 10 sec in order to avoid damage the paw and the cut-off was set at 20 sec to avoid tissue damage. The test was repeated three times within 30 min.

**Biochemical parameters assessment**

**Blood glucose levels:** Blood glucose levels were estimated by glucose oxidase-peroxidase method.

**GSH estimation:** The sciatic nerve homogenate was used for the estimation of GSH. The sciatic nerve was collected from rats. Then, the nerve was homogenized in phosphate buffer saline (pH 7.4). Aliquot
(60 μL) of homogenate was mixed with 60 μL of 1% trichloroacetic acid (TCA) solution and kept at 4°C for 30 min. The resulting solution was centrifuged at 1000 rpm for 5 min at 4°C. The supernatant was used for the estimation of GSH content as method.

**Lipid peroxidation:** Lipid peroxidation was estimated by measuring the formed thiobarbituric acid reactive species (TBARS) at 532 nm by colorimeter (Zhang et al., 2004).

**SOD activity:** SOD activity was measured by using commercial available assay kit (Cat. No. CS 19160).

**Catalase activity:** Catalase activity was measured with commercial available assay kit (Cat. No. CS 100). Sciatic nerve homogenate was used for the estimation of SOD and catalase activity.

**Nitrite estimation:** Plasma nitrite levels were estimated by using Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloric acid in water) which served as an indicator of nitric oxide production. At the end of the study, the animals were sacrificed under mild anaesthesia and blood was collected from jugular vein in EDTA tubes. The plasma was separated by using cold centrifuge at 2500 rpm for 10 min. One hundred microliter of separated plasma was mixed with 500 μL of Greiss reagent and incubated at room temperature for 10 min. Absorbance was measured at 546 nm spectrophotometrically. The nitrite concentration was calculated from standard curve using sodium nitrite as standard and expressed as μ mole of nitrite/litre (Green et al., 1982).

**Statistical analysis:** All the results expressed as Mean±SEM, significance of difference between multiple groups was evaluated using one-way analysis of variance (ANOVA). When ANOVA showed significant difference, post hoc analysis was performed with Dunnett's test. p<0.05 was considered statistically significant. Statistical analysis was carried out by using Graph Pad, Prism software.

**RESULTS**

**Effect of *O. corniculata* on blood glucose levels and body weight:** STZ-induced diabetic rats were showed approximately greater than four folds significant increase in blood glucose levels (Table 1) compared with age-matched control group after STZ injection (50 mg kg⁻¹). Chronic treatment with EEOC from 5th to 8th week resulted in slightly decreased in blood glucose levels and protected from decrease in body weight.

**Effect of *O. corniculata* on thermal hyperalgesia:** At the end of the 5th week, Diabetic rats were showed significant reduction in tail flick latency by 49% in hot water tail immersion test (Table 2) and decreased paw withdrawal response by 47% in hot plate test (Table 3) from control group. Treatment with two doses (200, 400 mg kg⁻¹) of EEOC for four weeks (from 5th to 8th week) were produced a significant increase in tail flick latency and paw withdrawal in diabetic rats in dose dependent manner. Maximum protective effect was observed after 4 weeks treatment with EEOC at the dose 400 mg kg⁻¹.

**Effect of Oxalis corniculata on GSH:** Diabetic rats showed significant decrease in GSH levels (Table 4) in sciatic nerve as compared with age matched control group. Treatment with EEOC (400 mg kg⁻¹) showed a significant increase in GSH levels but not significant at 200 mg kg⁻¹.

Table 1: Effect of EEOC on body weight and blood glucose levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Initial blood glucose levels (mg dL⁻¹)</th>
<th>Final blood glucose levels (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>231.8±12.15</td>
<td>279.1±25.24</td>
<td>107.3±3.73</td>
<td>115.6±2.94</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>230.6±10.74</td>
<td>158.6±48.38</td>
<td>437.6±14.25</td>
<td>520.1±10.49</td>
</tr>
<tr>
<td>EEOC 200 mg kg⁻¹</td>
<td>225.8±1.94</td>
<td>197.5±26.30</td>
<td>475.3±14.03</td>
<td>435.5±10.85**</td>
</tr>
<tr>
<td>EEOC 400 mg kg⁻¹</td>
<td>223.8±13.86</td>
<td>207.0±8.56</td>
<td>472.0±13.52</td>
<td>353.3±15.18</td>
</tr>
</tbody>
</table>

*p<0.01 compared with diabetic control group, ^p<0.01 compared with control group

Table 2: Effect of EEOC on hot plate method

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.87±0.09</td>
<td>8.87±0.05</td>
<td>8.82±0.05</td>
<td>8.94±0.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.59±0.08*</td>
<td>2.0±0.03*</td>
<td>2.0±0.04*</td>
<td>1.95±0.02*</td>
</tr>
<tr>
<td>EEOC 200 mg kg⁻¹</td>
<td>3.62±0.08***</td>
<td>4.01±0.08***</td>
<td>4.16±0.05***</td>
<td>4.75±0.05***</td>
</tr>
<tr>
<td>EEOC 400 mg kg⁻¹</td>
<td>5.04±0.04***</td>
<td>4.94±0.14***</td>
<td>4.16±0.04***</td>
<td>6.68±0.09***</td>
</tr>
</tbody>
</table>

*^p<0.001 compared with diabetic control, ^p<0.001 compared with control group*
Table 3: Effect of EEOC on hot water tail-immersion test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.8±0.12</td>
<td>10.9±0.07</td>
<td>10.7±0.04</td>
<td>10.6±0.16</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>2.5±0.59</td>
<td>2.06±0.06</td>
<td>2.06±0.041</td>
<td>2.04±0.04</td>
</tr>
<tr>
<td>EEOC 200 mg kg⁻¹</td>
<td>4.2±0.29**</td>
<td>4.14±0.11***</td>
<td>5.11±0.22***</td>
<td>5.36±0.22***</td>
</tr>
<tr>
<td>EEOC 400 mg kg⁻¹</td>
<td>5.5±0.47***</td>
<td>6.05±0.41***</td>
<td>7.18±0.29***</td>
<td>7.35±0.08***</td>
</tr>
</tbody>
</table>

***p<0.001, **p<0.01 compared with diabetic control, 'p<0.01 compared with control

Table 4: Effect of EEOC on Lipid peroxidation, GSH, SOD and Catalase activity in nerve homogenate of diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MDA (µM mg⁻¹ of protein)</th>
<th>GSH (µM mg⁻¹ of protein)</th>
<th>SOD (µU mg⁻¹ protein)</th>
<th>Catalase (pmole min mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5±0.29</td>
<td>50±73±0.97</td>
<td>4.15±0.58</td>
<td>30.16±0.71</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>22.4±0.56</td>
<td>23.5±0.62</td>
<td>2.78±0.22</td>
<td>11.09±0.44</td>
</tr>
<tr>
<td>EEOC 200 mg kg⁻¹</td>
<td>18.8±0.35**</td>
<td>25.29±0.44**</td>
<td>2.94±0.65**</td>
<td>16.47±0.64***</td>
</tr>
<tr>
<td>EEOC 400 mg kg⁻¹</td>
<td>12.8±0.59**</td>
<td>44.15±0.78**</td>
<td>3.12±0.52**</td>
<td>27.20±0.57***</td>
</tr>
</tbody>
</table>

***p<0.001, **p<0.01 compared with diabetic control, 'p<0.01 vs. control, ns = Not significant

Effect of Oxalis corniculata on lipid peroxidation: After 8 weeks of STZ injection, diabetic rats were showed significant increase in MDA levels (Table 4) in sciatic nerve as compared with age-matched control group. Treatment with two doses (200 mg kg⁻¹, 400 mg kg⁻¹) of EEOC for four weeks showed significant decrease in MDA in diabetic rats.

Effect of Oxalis corniculata on SOD activity: Sciatic nerve SOD activity was significantly decreased (Table 4) in 8 weeks. After 4 weeks treatment with EEOC was showed significantly increase in SOD activity in diabetic rats.

Effect of Oxalis corniculata on catalase activity: After 8 weeks of diabetic rats, catalase activity was significantly decreased (Table 4) as compared with the age-matched control rats. Four weeks treatment with EEOC 200 and 400 mg kg⁻¹ significantly inhibited the decrease in catalase activity in treated diabetic rats.

Effect of Oxalis corniculata on nitrite levels: Diabetic rats had shown significant increase in plasma nitrite levels as compared with age matched control rats (p<0.0001). Treatment with EEOC showed a significant reduction as dose dependant manner in plasma nitrite levels.

DISCUSSION

Diabetic neuropathy is evident from previous reports i.e., decreased motor and sensory nerve conduction velocity (MNCV and SNCV), mechanical and thermal hyperalgesia, allodynia and decreased nerve blood flow (NBF) (Negi et al., 2010; Sayyed et al., 2006). Mechanism underlying diabetic neuropathy is activation of increased aldose reductase activity, formation of advanced glycation end products (AGEs), activation of protein kinase C, activation of hexosamine pathway, poly (ADP-ribosylation) polymerase (PARP). Oxidative-nitrosative stress, activation of mitogen-activated protein kinase, 12/15-lipoxygenase activation and impaired neurotropic support (Crowe et al., 2008; Negi et al., 2010; Obrosova et al., 2001; Purves et al., 2001; Drel et al., 2007; Obrosova et al., 2001; Stavniuchik et al., 2010). All the above factors are important in the formation of free radicals which may cause neuronal damage.

In the present study, STZ-injected rats were shown significantly higher in blood glucose levels, decreased bodyweight and decreased nociceptive threshold to noxious stimulus as compared with non-diabetic control rats which indicating in diabetic rats exhibit thermal hyperalgesia. These results are similar to several previous reports (Calcott et al., 1994; Courteix et al., 1993; Negi et al., 2010; Chopra et al., 2010). The purpose of the present study was to evaluate the effect of Oxalis corniculata on neuropathic pain and oxidative stress in diabetic rats.

Diabetic nephropathy is the most common microvascular complications of hyperglycemia (Behnam-Rassouli et al., 2010). Neuropathic pain is a most common symptom associated with diabetic nephropathy. In the present study, we evaluated the nociceptive response to thermal noxious stimuli in diabetic rats. Nociception is evident from previous studies, have shown decreased tail flick latency and paw withdrawal from heat source in diabetic rats (Chopra et al., 2010; Sayyed et al., 2006). After treatment with EEOC, diabetic rats were shown increase in tail flick latencies and paw withdrawal as compared with vehicle treated diabetic rats. This could be due to multiple etiology of nociception in diabetic neuropathy.

Moreover, administration of O. corniculata attenuated increased lipid peroxidation, decreased levels of antioxidant enzymes (GSH, SOD and Catalase activity) in diabetic rats. Therefore, it may suggested that antioxidant action of O. corniculata is contributing to prevent reactive oxygen species (ROS) induced cellular damage in sciatic nerve of diabetic rats.
This condition is called oxidative stress. Several lines of evidences have shown in reactive oxygen species mediated oxidative stress in diabetic neuropathy (Cameron et al., 2001; Sayyed et al., 2006). Similarly, α-Lipoic acid, an antioxidant, has shown improved levels of antioxidant enzymes levels and corrects the deficits in diabetic neuropathy (Stevens et al., 2000). Oxidative stress is due to imbalance between production and neutralization of reactive oxygen species.

Important factors involved in hyperglycemia-induced oxidative injury to nerve are superoxide and nitric oxide. These forms a potent oxidant, peroxynitrite which is easily crosses cell membrane, causes the protein nitration or nitrosylation, lipid peroxidation and break the DNA strands leads to the cell death and has direct toxic effects on nerve tissue resulting neuropathic pain (Kim et al., 2003; Sampath et al., 2010). Peroxynitrite decomposition catalysts counteract the sensory neuropathy in STZ-induced diabetic experimental animals (Drel et al., 2007). In our study, we observed increased levels of plasma nitrite levels which indicated nitrosative stress in diabetic rats. Treatment with *Oxalis corniculata* was attenuated increased plasma nitrite levels, it may be due to its inducible NOS (iNOS) and action of peroxynitrite (Sengupta et al., 2006).

**CONCLUSION**

Present study shows that the administration of BEOC showed beneficial effects that including ameliorated effect in nociception, preventing diabetic complications from lipid peroxidation and oxidative stress in experimental STZ-induced diabetic neuropathy in rats. This effect might be due to preventing free radical production in diabetic rats. This could be helpful for prevention or early treatment of diabetic complication. However, further merit investigations including pharmacological and biochemical investigations to elucidate the mechanism of antioxidant and ameliorative action of the constituents of *Oxalis corniculata* plant are necessary.

**REFERENCES**


