EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF *OXALIS CORNICULATA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

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ABSTRACT

**Background:** *Oxalis Corniculata* is traditionally well known for its versatile uses. The present study was carried out to evaluate the antinociceptive action of ethanolic extract of *Oxalis corniculata* in Streptozotocin induced diabetic albino rats. **Method:** To look for the antinociceptive effect the albino rats were divided into 5 groups, each consisting of 6 animals. Diabetes was induced by a single i.p. injection of Streptozotocin at a dose of 50mg/kg body weight. Standard drug, Pentazocine was fed to one group in the hot plate model and Aspirin was fed as the standard drug in acetic acid induced writhing model. Ethanolic extract of *Oxalis corniculata* (EEOC) at doses 200mg/kg and 400mg/kg body weight was fed to the rats in the test groups. **Results:** The antinociceptive property of the extract has shown increasing trend with increase in dose of the extract. **Conclusion:** Results obtained in this study substantiate the anti-nociceptive activity of EEOC leaves in streptozotocin induced diabetic rats.

**KEYWORDS:** Antinociceptive; *Oxalis Corniculata*; Streptozotocin.

INTRODUCTION

Diabetes is one of the major chronic non-communicable metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels.[1] The two broad categories of diabetes mellitus are designated as type 1 and type 2. Type 1 diabetes mellitus results from autoimmune beta cell destruction, which leads to insulin deficiency. Type 2 diabetes mellitus is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production.
Although type 1 diabetes mellitus most commonly develops before the age of 30, autoimmune beta cell destruction can develop at any age. Type 2 diabetes mellitus develops more rapidly with increasing age, but it also occurs in children, particularly in obese individuals.\(^2\) The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025\(^3\) and the number of people with DM are set to rise from an estimate of 150 million in 2008 to 220 million in 2010 and 300 million in 2025.\(^4\) The countries with the largest number of diabetic people are and will be in the year 2025, India, China and United States.\(^3\) The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.\(^5\)

Painful Diabetic Neuropathy (PDN) may constitute a considerable clinical problem and a burdensome condition worldwide. The symptoms of painful diabetic neuropathy are highly unpleasant for the individuals and affect their quality of life.\(^6\) Various classes of drug are currently under investigation to treat neuropathic pain but still there is no gold standard therapeutic approach or treatment to manage this difficult to treat pain.\(^7\) Therefore there is an urgent need to search for some novel strategies based approaches or new drugs for alleviating PDN especially those with novel mechanism of action where a combination of beneficial and less side effect may be achieved. Pain caused by diabetic neuropathy is debilitating and often refractory to classical analgesics including morphine.\(^8\)

The chronic pain that follows peripheral nerve injury differs fundamentally from neuropathic pain and conventional analgesic are well reported to be partially effective or ineffective in neuropathic pain. Complementary medicines have gained popularity in recent years. Many indigenous medicinal herbs have been found to be useful to successfully manage pain in various chronic pain models.\(^9\)

Various classes of drugs are being examined and used for the treatment of neuropathic pain and strict glycemic control remains the best preventive measure for neuropathy.\(^10\) These classes of drugs include antidepressants, antiepileptics, NSAID’s, Opioids, Alpha lipoic acid, topical analgesics and also non pharmacological measures as physiotherapy, acupuncture and Transcutaneous electrical nerve stimulation (TENS).\(^11\) However more than 30-40 %patients are unable to achieve complete pain relief even after glycemic control.\(^12\)

*Oxalis corniculata* Linn. (Family- Oxalidaceae) also known as creeping wood sorrel grows at
Dutta et al. World Journal of Pharmacy and Pharmaceutical Sciences

subtropical climate, being native to India and especially abundant in the north-east. It is a herbaceous plant distributed in damp shady places, roadside, lawns. Phytochemical investigations of the plant have revealed presence of flavonoids, Vitamin-C, carotene, oxalates etc. It is used as an antihelminthic, anti inflammatory, analgesic, astringent, diuretic and stomachic. Some studies have shown the plant to have wound healing,[13] cardio relaxant,[14] anticancer, [15] antimicrobial, [16] antifungal, [17] anti implantation and abortifacient activity.[18]

The present study aims to evaluate the antinociceptive effect of different doses of ethanolic extract of *Oxalis corniculata* in Streptozotocin induced diabetic rats in both hot plate and acetic acid induced writhing model of analgesia.

MATERIALS AND METHODS

Ethical Review

The protocol was submitted to the Institutional Animal Ethics Committee of Gauhati Medical College and Hospital, Guwahati bearing CPCSEA Registration No. 351, 3/1/2001. It was approved by the Committee bearing approval no. MC/32/2013/3 and the CPCSEA guidelines were adhered to during the study.

Drugs and Chemicals Used In the Study

Ethanolic extract of *Oxalis corniculata* (EEOC), Streptozotocin (Obtained from Himedia, Mumbai), Normal Saline (0.9% NaCl), Gum acacia, Pentazocine Ampoule (30mg/ml. obtained from Ranbaxy Pharmaceuticals), Aspirin obtained from USV Ltd., India.

Plant Material

Whole plant of *Oxalis corniculata* Linn. was collected from in and around Guwahati, India. Authentication of the plant was done in the Department of Botany, Gauhati University and a voucher specimen was preserved for further reference. The whole plant was thoroughly washed, shade dried, and then chopped to a coarse powder using a mixer grinder. Powder (200 g) was tightly packed in Soxhlet apparatus and extracted employing ethanol as solvent for 5 days at a temperature of 40-60°C using a heating mantle. The extract was filtered using Whatman filter paper no.1 and the filtrate was evaporated on a water bath until it gets concentrated. The jelly-like extract of the leaves was collected in a petri dish. A final yield of 40.5 g was obtained. The percentage yield of *Oxalis corniculata* was 20.25% (w/w) with
respect to the original dried powder. The extract was stored in a refrigerator at 4°C in labeled air-tight containers for further use.

**Experimental Animals Used In the Study**

The study was carried out in healthy albino rats of Sprague Dawley variety of either sex weighing between 200-250 gm. procured from Animal House, Gauhati Medical College. The animals were fed on rat chaws diet and water *ad libitum* during the experiment. Animals were maintained under controlled condition with 12 hour light and 12 hour dark cycles at a temperature of 24 ± 1°C and humidity of 55 ± 5 %. Before conducting the experiment all the animals were acclimatized to laboratory condition for 7 days. The animals were housed in separate polypropylene cages inside a well ventilated room and their bedding changed from time to time.

**Acute toxicity study**

Acute toxicity study was done according to OECD 425 guidelines. The animals were found to be alive at 2000 mg/kg per oral feeding of the Ethanolic extract of *Oxalis corniculata* (EEOC).

**Induction of Diabetes in Rats**

The animals were fasted overnight prior to the induction of diabetes. Streptozotocin (Product code 1758) of Himedia Laboratories, India was dissolved in ice cold 0.1 M citrate buffer, pH 4.5 and always prepared freshly for immediate use within 5 min.\(^{[19]}\)

STZ injections were given i.p. in a single dose of 50 mg/kg body wt. and the doses were determined according to the body weight of animals. In the present study the blood glucose levels were evaluated on Day 0 in all the rats prior to administration of Streptozotocin. On Day 3 i.e. after 72 hours, the blood glucose levels were evaluated and the rats with blood glucose level >250 mg/dl were considered as diabetic and taken up for the study. Pascoe and Storlien\(^{[20]}\) in 1990 mentioned that in general, rats are considered diabetic if tail blood glucose concentrations are greater than 200–300 mg/dl, 2 days after STZ injection.

**ANTINOCICPEPTIVE TESTING**

The antinociceptive activity of ethanolic extract of *Oxalis corniculata* Linn. was tested in albino rats by the following methods:

1) Hot plate test as described by Eddy and Leimbach (1953).\(^{[21]}\)
2) Acetic acid induced writhing test as described by Koster et al. (1959).[22]

**HOT PLATE TEST**

**MATERIALS**

**Animals:** Albino rats

**Drugs:** Pentazocine (Dose 10mg/kg i.p.) Each ampoule contains 30 mg/ml. Stock solution is prepared by diluting in distilled water and the dose per rat as per its weight is calculated in terms of I.U. (Insulin units) and given to Nociceptive standard diabetic rats.

Ethanolic extract of *Oxalis corniculata* – Administered to GROUP IV and V after diluting in 2% carboxymethylcellulose.

**Equipments:** Eddy’s hot plate analgesiometer, Insulin syringe, Stop watch.

**METHOD**

The surface of the hot plate measured 25.3 cm², and was surrounded by 30 cm high Plexiglas walls. Each rat was placed on the Eddy’s hot plate which was kept at constant temperature of 55°C ± 1°C and the withdrawal latency was noted by observing either the licking of the hind paws, jumping or the rotation movements at 30th, 60th and 90th minute after `standard drug i.e. Pentazocine administration and also the two doses of extract administration. Withdrawal latency in seconds was measured and an increase in time interval was indicative of analgesia. A cut off time of 20 seconds was used to avoid tissue injury.[23]

**EXPERIMENTAL DESIGN FOR ANTINOCICEPTIVE STUDY (HOT PLATE METHOD)**

The animals included in the experiment were divided as follows:

**GROUP I:** NORMAL CONTROL GROUP: Received Normal Saline in the dose of 10ml/kg/day per orally.

**GROUP II:** DIABETIC CONTROL GROUP: Received Normal Saline in the dose of 10ml/kg/day per orally.

**GROUP III:** NOCICEPTIVE STANDARD GROUP: Received Pentazocine at a dose of 10 mg/kg per orally.

**GROUP IV:** DIABETIC TEST: Received Ethanolic extract of *Oxalis corniculata* 200mg/kg/day per orally.
GROUP V: DIABETIC TEST: Received Ethanol extract of *Oxalis corniculata* 400mg/kg/day per orally.

6 diabetic rats were included in all the groups except GROUP I which was the normal control group.

**ACETIC ACID INDUCED WRITHING TEST**

**Materials**

**Animals:** Albino rats

**Drugs:**

**Aspirin** - Administered to **GROUP III** at a dose of 400mg/kg. [24] Aspirin is dissolved in gum acacia and then administered per orally to the rats.

**Acetic acid** 1% v/v (1ml/100 gm of body weight of the animal).

Ethanol extract of *Oxalis corniculata* – Administered to **GROUP IV** and **V** after diluting in 2% carboxymethylcellulose.

**Equipments**

Beaker, Disposable syringe, Bell jar, Stop watch.

**Method**

After the administration of standard drug and the extract doses, writhing was induced 20 minutes later in each group by administering appropriate volume of acetic acid solution. The onset of writhes is noted. The number of abdominal contractions, trunk twist response and extension of hind limbs is counted for a period of 20 minutes. [25]

**EXPERIMENTAL DESIGN FOR ANTINOICEPTIVE STUDY (ACETIC ACID INDUCED WRITHING METHOD)**

The animals included in the experiment were divided as follows:

**GROUP I**: NORMAL CONTROL GROUP: Received Normal Saline in the dose of 10ml/kg/day per orally.

**GROUP II**: DIABETIC CONTROL GROUP: Received Normal Saline in the dose of 10ml/kg/day per orally.

**GROUP III**: NOCICEPTIVE STANDARD GROUP: Received Aspirin at a dose of 400 mg/kg per orally.

**GROUP IV**: DIABETIC TEST: Received Ethanol extract of *Oxalis corniculata* 200mg/kg/day per orally.
GROUP V: DIABETIC TEST: Received Ethanolic extract of *Oxalis corniculata* 400mg/kg/day per orally.

The rats in GROUP I, GROUP II, GROUP IV, GROUP V (6 Rats in each group) were the same that were used to look for the hot plate method. However in GROUP III that was the Nociceptive standard group, 6 diabetic rats were included and treated with Aspirin 400mg/kg per orally.

STATISTICAL ANALYSIS
The statistical analysis was carried out using Graph pad prism 5.01 software. Data were expressed as mean ± SEM. Results were analyzed by one way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. p value < 0.05 was considered as statistically significant.

RESULTS AND OBSERVATIONS
TABLE 1 and GRAPH 1 shows the effect of ethanolic extract of *Oxalis corniculata* Linn. in hot plate model of analgesia.

TABLE 1: Analgesic activity of Ethanolic extract of *Oxalis Corniculata* Linn. in Streptozotocin induced Diabetic rats using Hot Plate Method.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>REACTION TIME IN SECONDS (MEAN±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 MIN</td>
</tr>
<tr>
<td>GROUP I (NORMAL CONTROL)</td>
<td>9.50±0.428</td>
</tr>
<tr>
<td>GROUP II (DIABETIC CONTROL)</td>
<td>2.83±0.307^a</td>
</tr>
<tr>
<td>GROUP III (NOCICEPTIVE STANDARD PENTAZOCINE 10 mg/kg I.P.)</td>
<td>11.83 ± 0.307^b</td>
</tr>
<tr>
<td>GROUP IV DIABETIC TEST (EEOC200mg/kg)</td>
<td>4.66 ± 0.210^b</td>
</tr>
<tr>
<td>GROUP V DIABETIC TEST (EEOC400mg/kg)</td>
<td>7.16 ± 0.166^b</td>
</tr>
</tbody>
</table>

F 146.9 82.28 116.7
One way ANOVA df 4, 25 4, 25 4, 25
P <0.01 <0.01 <0.01

Values are expressed as Mean ± SEM (n=6);
One Way ANOVA followed by Dunnett’s multiple comparison tests is done. \(^a\) p<0.01 when compared to the Normal control group. \(^b\) p<0.01 when compared to the Diabetic control group.

![Graph 1: Hot Plate Test](image)

**GRAPH 1 HOT PLATE TEST**

At 30 minutes, the mean reaction time of Normal Control group was 9.50±0.428 sec, reaction time of Diabetic Control group was 2.83±0.307 sec, in the Nociceptive Standard group it was 11.83 ± 0.307 sec, in the Diabetic test (EEOC 200mg/kg) it was 4.66 ± 0.210 sec. and in the Diabetic test (EEOC 400mg/kg) it was 7.16 ± 0.166 sec.

At 60 minutes, the mean reaction time of Normal Control group was 9.33±0.421 sec, reaction time of Diabetic Control group was 3.33±0.333 sec, in the Nociceptive Standard group it was 12.50±0.562 sec, in the Diabetic test (EEOC 200mg/kg) it was 4.83±0.307 sec. and in the Diabetic test (EEOC 400mg/kg) it was 6.66 ± 0.333 sec .

At 90 minutes, the mean reaction time of Normal Control group was 9.66±0.557 sec, reaction time of Diabetic Control group was 3.66±0.210 sec, in the Nociceptive Standard group it was 13.50±0.428 sec, in the Diabetic test (EEOC 200mg/kg) it was 5.33±0.210 sec. and in the Diabetic test (EEOC 400mg/kg) it was 7.50 ± 0.223 sec.

It can be inferred that at 30, 60 and 90 min the reaction time in sec. i.e. withdrawal latency was maximum with the Nociceptive Standard i.e. Pentazocine, followed by Diabetic Test (EEOC 400mg/kg) and then Diabetic Test (EEOC 200 mg/kg). An increase in time interval was indicative of analgesia. One way ANOVA of the data when done the p value was found to be significant. It was followed by Dunnett’s test. There was significant difference in between the groups when Normal Control and Diabetic Control were taken as the control.
column. So the analgesic effect of the extract can be said to have increased in a dose dependent manner.

**TABLE 2** and **GRAPH 2** shows the effect of ethanolic extract of *Oxalis corniculata* Linn. in acetic acid induced writhing model of analgesia.

**TABLE 2- Analgesic activity of Ethanolic extract of Oxalis Corniculata Linn. on Acetic Acid induced writhing response in Streptozotocin induced Diabetic rats.**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>NUMBER OF WRITHING MOVEMENTS(MEAN± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I (NORMAL CONTROL)</td>
<td>67.83 ± 0.833</td>
</tr>
<tr>
<td>GROUP II (DIABETIC CONTROL)</td>
<td>76.66 ± 0.881a</td>
</tr>
<tr>
<td>GROUP III (NOICEPTIVE STANDARD ASPIRIN 400mg/kg P.O.)</td>
<td>30.33 ± 0.843b</td>
</tr>
<tr>
<td>GROUP IV DIABETIC TEST (EEOC 200mg/kg.)</td>
<td>51.83 ± 1.166b</td>
</tr>
<tr>
<td>GROUP V DIABETIC TEST (EEOC 400mg/kg.)</td>
<td>41.83 ± 0.600b</td>
</tr>
</tbody>
</table>

One way ANOVA    F  453.7    df  4, 25    p <0.01
Values are expressed as Mean ± SEM (n=6);

One Way ANOVA followed by Dunnett’s multiple comparison tests is done. a p<0.01 when compared to the Normal control group. b p<0.01 when compared to the Diabetic control group.

**GRAPH 2: ACETIC ACID INDUCED WRITHING TEST**
The mean number of writhing movements with the Normal Control group was found to be 67.83 ± 0.833, with the Diabetic Control group it was found to be 76.66 ± 0.881, with the Nociceptive Standard Aspirin it was found to be 30.33 ± 0.843, with the Diabetic Test (EEOC 200mg/kg) it was 51.83 ±1.166 and with the Diabetic test (EEOC 400mg/kg) it was found to be 41.83 ± 0.600. One way ANOVA of the data when done the p value was found to be significant. It was followed by Dunnett’s multiple comparison tests. There was significant difference in between the groups when Normal Control and Diabetic Control were taken as the control column. The number of writhing was significantly reduced in both the test groups and the standard group. It was minimum with the Standard drug, followed by high dose of the extract and then the lower dose. So the antinociceptive effect of the extract can be said to have increased in a dose dependent manner.

DISCUSSION

Diabetic neuropathy is one of the most painful complications of diabetes mellitus and upto 30% of patients with diabetes mellitus develop diabetic neuropathy. An interesting observation is seen in India, the prevalence of diabetic neuropathy is around 19% i.e., every fifth individual in the population, particularly among known diabetics, has a chance of developing this microvascular complication. The mechanism underlying diabetic neuropathic pain are complex and both peripheral and central components of sensory systems are reported to be involved in progression and maintenance of neuropathy. The ideal approach for treating diabetic neuropathy is to maintain the normoglycemia in diabetic patients throughout life span which is practically impossible as the best clinically proved medicine provides only symptomatic relief. Hence, there is definitely a need for finding specific treatment.

In the study to look for the antinociceptive effect both the central and peripheral models of analgesia were used. The hot plate method was used to look for the central mechanism of analgesia, whereas to look for the peripheral mechanism acetic acid induced writhing response was used.

It was seen that at 30, 60 and 90 min. the reaction time in sec. i.e. withdrawal latency was maximum with the Nociceptive Standard Pentazocine, followed by Diabetic Test (EEOC 400mg/kg) and then Diabetic Test (EEOC 200 mg/kg). An increase in time interval was indicative of analgesia. With increase in dose of the extract it was seen that the reaction time was increased. However the extract at both the doses were not able to increase the reaction
time to the level of the standard. Thus it can be said that the extract had antinociceptive property in this model of analgesia. Previous studies on other plant extracts have also made use of the fact that diabetic rats exhibit thermal hyperalgesia.

In the peripheral model, acetic acid was injected i.p. which resulted in writhing response in the rats due to the irritant nature of acetic acid. The number of writhing was significantly reduced in both the test groups and the standard group. It was minimum with the Standard drug, Aspirin followed by high dose of the extract and then the lower dose. So the analgesic effect of the extract can be said to have increased in a dose dependent manner. However the extract could not produce as much effect as the standard drug Aspirin. The abdominal contraction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that Oxalis corniculata exerts its analgesic effect probably by inhibiting action of prostaglandins.\(^{28}\)

Previous phytochemical analysis on various plants used as an analgesic have revealed that one of the largest groups of chemicals present are the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications. The presence of alkaloids in Oxalis corniculata might be responsible for its antinociceptive propery.

Khamkar P A et al\(^{28}\) had carried out a study wherein the analgesic and anti-inflammatory effect of Oxalis corniculata was seen. The results of the present study run parallel to the results of that study wherein with increase in dose of the extract the antinociceptive property also increased.

V. Sampath Kumar et al (2012)\(^{29}\) evaluated the ethanolic extract of Oxalis corniculata at doses of 200 and 400mg/kg body weight for its antinociceptive activity in diabetic neuropathy rats. Diabetic rats showed significant reduction in tail flick latency by 49% in hot water tail immersion test and decreased paw withdrawal by 40% in hot plate test by the end of 5\(^{th}\) week.

Further investigations are required to find active component of the extract and to confirm the mechanism of action responsible for antinociceptive effect.

Conflict of interest: None declared.

Ethical approval: The study was approved by the Institutional Animal Ethics Committee.
REFERENCES


