Neuroprotective role of *Pterocarpus marsupium* Roxb in streptozotocin-induced diabetic neuropathic pain in Type 2 diabetic rats

Venkatesh Gunasekaran*, Merin Maria Mathew, Mrinmoy Gautam, M. Ramanathan

**INTRODUCTION**

Traditional medicines are generally derived from medicinal plants, minerals, and organic sources. Usage of herbal medicine in developing and developed countries has been increased nowadays due to their therapeutic benefits and less side effects. One of the most common diabetic complication is diabetic neuropathy that occurs in approximately half of patients with diabetes. Diabetic neuropathy is characterized by progressive degeneration and impaired regenerative ability of peripheral nerve fibers, resulting in the progressive loss of the longest nerve fibers innervating the distal limb. Despite the fact that the availability of modern and herbal medicine increased frequently in treating diabetes, yet the treatment of diabetes complications remains a substantial challenge. Many studies revealed that controlled glycemia might reduce the risk of diabetes and diabetic complications, but few studies have reported that even with intensive insulin therapy or with conventional therapy, the incidence of neuropathy increased in patients with diabetes. Various pre-clinical studies have also reported the beneficial effects of herbal products in the management of diabetic neuropathy such as *Hypericum perforatum*, *Olea europaea*, *Curcuma longa*, *Emblica officinalis*, and some clinical reports also believed to control the in neuropathic pain by the drugs of plant origin. *Pterocarpus marsupium* Roxb (PM) (from the family: *Leguminosae*) known to have antiinflammatory, astringent, antacid, treatment of toothache, antiarthritic, antiarthritis, antiquot, antiasthmatics, antihyperlipidemic, and treating bronchitis and skin infections. PM is extremely rich in alkaloids and saponins possessing potential biological significance (Sharifi *et al.*, 2003). Aqueous

**ABSTRACT**

**Aim:** The present study was aimed to evaluate the neuroprotective effects of aqueous extract of *Pterocarpus marsupium* Roxb (PM) on the pain threshold response in streptozotocin (STZ)-induced diabetic neuropathic pain. **Material and Methods:** STZ (40 mg/kg; i.p.) administered for 4 weeks in rats was monitored in 0th and 8th week by measuring blood sugar levels and body weight. Thermal hyperalgesia, mechanical hyperalgesia, and thermal allodynia were performed in 0th, 4th, 6th, and 8th week of the study. At the end of 8th week study, formalin-evoked pain model followed by measurement of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 level, and morphological changes in sciatic nerve were studied. All the rats except the vehicle-treated group received insulin 1 IU/kg/day to maintain plasma glucose levels. **Results and Discussions:** After 4 weeks of diabetic induction, administration of PM (100 and 200 mg/kg) along with 1 IU/day for 4 weeks had no significant effect on body weight and feed intake when compared to control rats. Pregabalin (10 mg/kg) and PM (100 mg/kg and 200 mg/kg) were injected for 4 weeks significantly attenuated the nociception in behavioral models. Furthermore, pregabalin and PM significantly inhibited the TNF-α, IL-1β, and IL-6 levels comparable to STZ group. In comparison to STZ with insulin-treated rats, PM exhibited significant increase in the pain threshold response. PM also reversed the STZ-induced axonal degeneration and deposition of collagen fibers in the sciatic nerve. **Conclusion:** The study concludes that neuroprotective effect of PM in STZ-induced neuropathic pain can be attributed to its anti-inflammatory and neuroregeneration mechanism.

**KEY WORDS:** Histopathology, Inflammatory marker, *Pterocarpus marsupium* Roxb, Sciatic nerve, Streptozotocin
extracts of PM have been reported for their anti-inflammatory and antioxidant properties in in vivo and in vitro model.\textsuperscript{[11,12]}

In patient with Type 2 diabetes mellitus has been observed that elevated levels of TNF-\(\alpha\), IL-1\(\beta\), and IL-6 which is named as circulating cytokines by the early activation of innate immune system and chronic systemic inflammation.\textsuperscript{[13]} A variety of stressors such as infection, tissue injury and food causes macrophages, adipocytes, and endothelial cells, to secrete inflammatory cytokines.\textsuperscript{[14]} In this view, development of a drug which modulates the cytokines in Type 2 diabetes would be a novel approach in early intervention of the disease. By virtue of the previous reports, the present study was aimed to evaluate the role of PM on behavioral, inflammatory mediators, and morphological changes against streptozotocin (STZ)-induced diabetic neuropathy in rats.

MATERIALS AND METHODS

Preparation of PM and Drug Solutions

PM (Leguminosae), Malabar kino, Indian kino tree, or vijayasar is a plant drug belonging to the group called rasayana in Ayurvedic system of medicine. PM capsules were obtained from VASU Healthcare Pvt. Ltd, Gujarat. Each capsule contains 300 mg bark extract of PM and dissolved in distilled water. Phytochemical analysis was performed for the conformation of the presence of saponins, tannins, triterpenes, flavonoids, and alkaloids. STZ (catalog no. S0130) was procured from Sigma, USA. Primers of TNF-\(\alpha\), IL-1\(\beta\), and IL-6 were procured from sigma, USA. All other chemicals of analytical grade were purchased from Super Religare Laboratories, India.

Animals and Induction of Diabetic Neuropathy

Male Wistar rats (220-250 g) were obtained from the animal house, PSG Institute of Medical Sciences and Research (PSG IMS&R), Coimbatore. The rats were grouped and housed \((n = 6\) per cage) in a separate room with controlled temperature of 25°C ± 2°C, and a reversed light-dark cycle (12 h/12 h), and they had free access to food and water ad libitum. Animals were acclimatized to laboratory condition for 1 week before experiments. The experimental protocols were approved by the Institutional Animal Ethics Committee of PSG IMS&R, and experiments were performed according to the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals, Government of India.

Diabetes was induced by a single intraperitoneal injection of STZ (40 mg/kg; i.p) freshly dissolved in citrate buffer having pH 4.4 to overnight fasted rats. The STZ-treated rats were given 5% w/v glucose in drinking water for 24 h. Three days later, diabetes was confirmed by measuring the fasting blood glucose level. Animals showed fasting blood glucose >300 mg/dl was considered as diabetic and grouped. Fasting blood glucose and body weight were measured every week. The treatment was started after 4 weeks of STZ administration.\textsuperscript{[15]}

Body Weight and Fasting Blood Glucose

Body weight and fasting blood glucose were measured weekly. Fasting blood glucose was measured by true result glucometer.

Behavioral Experiments

Hot plate

The Eddy’s hot plate will be carried out by placing each animal in the hot plate apparatus having temperature maintained to 55°C ± 1°C. The cutoff time will be adjusted to 10 s to avoid the damage of the paw. The latency of paw licking or jumping responses are noted, and then animals were removed from the hot plate.\textsuperscript{[9]}

Electronic von frey test

Force was applied with 150 g on the interplantar region of the rat paw. The sudden raise and licking of the paw were considered as the threshold for pain. The force was applied with tips for minimum of three times and maximum of 10 times.\textsuperscript{[16]}

Randell–Selitto test

Mechanical hyperalgesia was assessed with the Randall and Selitto test using a paw pressure analgesiometer, which applies a linearly increasing mechanical force to the dorsum of the rat’s hind paw. The mechanical nociceptive threshold was defined as the force in grams at which the rat withdrew its paw. The constant force of 20 g/s. Amount of force applied was recorded. A cutoff time of 30 s was maintained.\textsuperscript{[17]}

Formalin test

At the end of 8\textsuperscript{th} week study, all the groups were subjected to formalin test. Respective treatments were given to the corresponding groups 1 h before the formalin test. Each animal was acclimatized to the observation box before the formalin test. After an adaptation period of 15 min, the right hind foot paw was injected with 50 ml of 2.5% formalin in the intraplantar region. Nociception was evaluated by quantifying paw licking time and paw elevation time during the first 10 min (acute phase) and at 20-40 min (delayed phase).\textsuperscript{[18,19]}

Measurement of Inflammatory Cytokines

The RNA was isolated from the nerves with the help of TRI Reagent as given in the Technical Bulletin by
Sigma-Aldrich for RNA isolation. After dissolving, the RNA in 25-35 μl of DEPC water or Milli-Q water, we estimated the total RNA in NanoDrop 2000C (Thermo Scientific) in ng/μl. A total of 2 μg RNA was converted to cDNA with the help of Applied Biosystems cDNA conversion kit for a reaction of 20 μl in which MasterMix comprises 10 μl and Total RNA 10 μl (volume made up with Milli-Q or DEPC water). Then, after making the reaction for 20 μl with 10 μl MasterMix from NovaTaq Merck Kit and 1-2 μl cDNA volume made up to 10 μl with Milli-Q water. With 95°C for 10 min and keeping 60-65°C as annealing with 35 cycles and finally keeping an elongation time of 72°C for 1-5 min reaction is set up in ThermoMixer. Details of the primers and its sequence are given in Table 1.

**Histological Examination**

The histological examination of the myelin sheath level in the sciatic nerve was carried out to assess the extent of nerve degeneration. Samples of sciatic nerve were stored in the fixative solution (10% formalin), and staining was done using hematoxylin and eosin as described.[30]

**Statistical Analysis**

Data were given in ± standard error of the mean. The collected data were subjected to one-way ANOVA for behavior models followed by post hoc analysis Tukey’s multiple comparison test. Two-way ANOVA followed by Bonferroni post-test was used for comparing body weight and fasting blood glucose. *P*< 0.05 was considered as statistically significant. The analysis was carried out using GraphPad Prism software of version 5.03.

### Table 1: Details about the primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer/Sequence</th>
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<tr>
<td>GAPDH</td>
<td>Forward - CAACCTTGCCATCGTGGAAG</td>
</tr>
<tr>
<td></td>
<td>Reverse - CTGCCTCACCCACCTTCTT</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward - TCCCAACAAGGAGGAAGTTCC</td>
</tr>
<tr>
<td></td>
<td>Reverse - GGAGCCGCTCTCCCTGAAGAGA</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Forward - ACCAGCTTTCGACAGTGGAGGAGA</td>
</tr>
<tr>
<td></td>
<td>Reverse - TCTCCACAGCCACAAGGTGAGCA</td>
</tr>
<tr>
<td>IL-6</td>
<td>Forward - AGGATACACTTCCCAACAGACCT</td>
</tr>
<tr>
<td></td>
<td>Reverse - CAAGTGACATCGTTGTTAC</td>
</tr>
</tbody>
</table>

### Table 2: Effect of *Pterocarpus marsupium* on body weight and fasting blood

<table>
<thead>
<tr>
<th>Glucose in diabetic neuropathic pain model</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Column 2</th>
<th>Body weight (g)</th>
<th>Column 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>0&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>8&lt;sup&gt;th&lt;/sup&gt; week</td>
</tr>
<tr>
<td>STZ (diabetic control)</td>
<td>267.83±15.26</td>
<td>267.33±10.19</td>
<td>250.5±11.66</td>
<td>217.33±11.78</td>
</tr>
<tr>
<td>STZ+insulin (1 IU/day)</td>
<td>252±12.25</td>
<td>147.5±12.05***</td>
<td>242.83±12.4</td>
<td>230.33±10.52</td>
</tr>
<tr>
<td>STZ+pregabalin (10 mg/kg)</td>
<td>254±13.68</td>
<td>136.83±8.68</td>
<td>246.67±12.52</td>
<td>252.17±9.85</td>
</tr>
<tr>
<td>STZ+PM (100 mg/kg)</td>
<td>255.5±19.62</td>
<td>136.67±8.2</td>
<td>245.83±13.2</td>
<td>231.5±12.66</td>
</tr>
<tr>
<td>STZ+PM (200 mg/kg)</td>
<td>251.17±15.30</td>
<td>129±6.26**</td>
<td>249±12.1</td>
<td>228.83±8.59</td>
</tr>
</tbody>
</table>

All data expressed as mean±SD, *n* = 6. **P<0.001** denotes statistical significance as compared to diabetic control rats. ***P<0.001** denotes statistical significance as compared to insulin-treated diabetic rats. STZ: Streptozotocin, SD: Standard deviation

### RESULTS

#### Effect of PM on Body Weight

After 4 weeks of STZ injection in diabetic rats, insulin was administered (1 IU/day) for the next 4 weeks period. No significant changes in body weight and feed intake were observed in insulin-treated rats when compared with non-diabetic control group. Pregabalin had no significant effect on body weight and feed intake when compared with non-diabetic control rats. After 4 weeks of diabetic induction, administration of PM (100 and 200 mg/kg) along with 1 IU/day for 4 weeks had no significant effect on body weight and feed intake when compared to control rats (Table 2).

#### Effect of PM on Fasting Blood Glucose

Insulin administration for 4 weeks (4<sup>th</sup>-8<sup>th</sup> week) in diabetic rats significantly reduced the fasting plasma glucose level (*P< 0.001*) when compared with diabetic control rats. However, the glucose level was not brought to the baseline. Pregabalin treatment along with 1 IU insulin/day administration on comparison to insulin-treated rats, no significant reduction in the fasting blood glucose levels was observed. Effect of PM on plasma blood glucose was assessed by administering PM 100 and 200 mg/kg in diabetic insulin-treated rats. The results indicate that PM at doses of 200 mg/kg decreased the plasma glucose level in comparison to diabetic insulin-treated rats. The reduction in blood glucose level was observed at the 8<sup>th</sup> week (Table 2).

#### Effect of PM on Hot Plate Method

Treatment of insulin from 4<sup>th</sup> week, resulted in recovery of hyperalgesic response (*P< 0.001*) as observed by decreased hyperalgesic response (increased basal latency period), was observed to significant as compared to diabetic control on the 8<sup>th</sup> week (Figure 1). At the end of the 8<sup>th</sup> week treatment of pregabalin along with insulin in diabetic animals exhibited decrease in pain threshold response to thermal stimuli as compared to diabetic control (*P< 0.001*) and insulin-treated control (*P< 0.001*). However, even in the 6<sup>th</sup> week pregabalin-treated rats showed increased basal latency and had a significant difference in pain threshold level between diabetic control (*P< 0.001*)...
and diabetic insulin-treated diabetic rats ($P < 0.001)$ (Figure 1). PM was administered at two doses from 4th week showed significant increase in pain threshold. PM at dose of 100 and 200 mg/kg increased basal latency as compared to diabetic control ($P < 0.001$) at 6th and 8th week and insulin-treated diabetic control at 6th ($P < 0.01$) and 8th week ($P < 0.01$) (Figure 2).

**Effect of PM on Electronic von Frey**

Treatment of insulin in diabetic rats resulted in recovery of allodynia response ($P < 0.001$) as observed and was significant as compared to diabetic control on the 8th week (Figure 3). In the 6th and 8th week, pregabalin-treated rats showed reversed the allodynic response and had a significant difference in response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic rats ($P < 0.001$) (Figure 3). PM was administered at two doses from 4th week showed significant increase in allodynic response. PM at dose of 100 mg/kg increased the response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic control ($P < 0.001$) at 6th week. Further, in 8th week, it also increased the response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic control ($P < 0.001$). PM at dose of 200 mg/kg increased the response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic control ($P < 0.001$) at 6th week. Further, in 8th week, it also increased the response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic control ($P < 0.001$) (Figure 4).

**Effect of PM on Randell–Selitto Method**

Treatment of insulin from 4th week onward resulted in no recovery of mechanical hyperalgesic response as observed (Figure 5). In the 6th and 8th week pregabalin-treated rats showed reversed the mechanical hyperalgesic response and had a significant difference in response as compared to diabetic control ($P < 0.001$) and insulin-treated diabetic rats ($P < 0.001$) (Figure 5). PM at dose of 100 mg/kg increased the response as compared to diabetic control ($P < 0.05$) but had no significant difference as compared to insulin-treated diabetic control at 6th week. Further, in 8th week, it also increased the response as compared to diabetic control ($P < 0.01$) and insulin-treated diabetic control ($P < 0.01$). PM at dose of 200 mg/kg increased the response as compared to diabetic control ($P < 0.01$) and insulin-treated diabetic control ($P < 0.05$) at 6th week. Further, in 8th week, it also increased the response as compared to diabetic control ($P < 0.05$) and insulin-treated diabetic control ($P < 0.05$) (Figure 6).

**Effect of PM on Formalin Test in Rats**

**On acute phase (0-10 min)**

In acute phase, pregabalin (10 mg/kg)-treated rats showed no significant reduction in paw licking/biting time as compared to diabetic control rats. Treatment of PM showed significant reduction in paw licking/biting at 200 mg/kg in comparison to diabetic control rats ($P < 0.01$) and insulin-treated diabetic control rats ($P < 0.05$). Treatment of pregabalin, PM 100 and 200 mg/kg did not show significant difference in paw elevation time as compared with diabetic control and insulin-treated diabetic control rats (Figure 1a and b).

**On delayed phase (20-40 min)**

In delayed phase, pregabalin (10 mg/kg)-treated rats showed significant reduction in paw licking/biting time as compared to diabetic control rats ($P < 0.01$) and insulin-treated diabetic control rats ($P < 0.01$). PM at the dose (100 mg/kg) administered rats showed no significant reduction in paw licking/biting time. PM at the dose (200 mg/kg) administered rats showed significant reduction in paw licking/biting time in comparison to diabetic control ($P < 0.01$) and insulin-treated diabetic control rats ($P < 0.01$). In delayed phase, pregabalin (10 mg/kg)-treated rats showed significant reduction in paw elevation time as compared to diabetic control rats ($P < 0.001$) and insulin-treated diabetic control rats ($P < 0.01$). PM
at the dose (100 mg/kg) administered rats showed no significant reduction in paw elevation time. PM at the dose (200 mg/kg) administered rats showed significant reduction in paw elevation time in comparison to diabetic control \((P < 0.01)\) and insulin-treated diabetic control rats \((P < 0.05)\) (Figure 3a and b).

**Effect of PM on TNF-\(\alpha\) Level in Sciatic Nerve**

Four weeks of treatment with pregabalin (10 mg/kg) significantly decreased \((P < 0.001)\) the TNF-\(\alpha\) levels in diabetic rats in comparison to STZ and STZ with insulin-treated rats. STZ with insulin significantly decreased \((P < 0.05)\) the TNF-\(\alpha\) in comparison to STZ alone-treated rats. PM (100 and 200 mg/kg) treatment with insulin significantly decreased \((P < 0.05,\ P < 0.001)\) the TNF-\(\alpha\) levels in comparison to STZ with insulin-treated group. PM (100 and 200 mg/kg) treatment along with insulin significantly decreased \((P < 0.01,\ P < 0.001)\) the TNF-\(\alpha\) levels in comparison to STZ alone-treated group (Figure 5a).

**Effect of PM on TIL-1\(\beta\) Level in Sciatic Nerve**

There were no significant changes in sciatic nerve IL-1\(\beta\) level between the STZ and STZ insulin-treated group. Pregabalin (10 mg/kg) significantly decreased the IL-1\(\beta\) level as compared to STZ and STZ insulin-treated group. PM (100 and 200 mg/kg) treatment with insulin significantly decreased \((P < 0.05,\ P < 0.001)\) the IL-1\(\beta\) levels in comparison to STZ alone-treated group. In the same way, PM (100 and 200 mg/kg) treatment with insulin significantly decreased \((P < 0.05,\ P < 0.001)\) the IL-1\(\beta\) levels in comparison to STZ insulin treated group (Figure 5b).

**Effect of PM on IL-6 Levels in Sciatic Nerve**

Significant reduction of IL-6 level was observed for pregabalin (10 mg/kg) in compared to STZ and STZ insulin-treated group. Between the STZ and STZ insulin-treated group no significant changes were observed \((P > 0.05)\). PM (200 mg/kg) treatment with insulin significantly decreased \((P < 0.01)\) the IL-6 levels in comparison to STZ and STZ insulin-treated group. However, PM (100 mg/kg) treatment with insulin did not show significant reduction in comparison to STZ insulin-treated group \((P > 0.05)\). In contrast, PM (100 mg/kg) treatment with insulin significantly decreased \((P < 0.01)\) the IL-6 levels in comparison to STZ-treated group (Figure 5c).

![Figure 2](image2.png)

**Figure 2:** Effect of *Pterocarpus marsupium* on thermal hyperalgesia in diabetic neuropathic pain model. Values are expressed as mean ± standard deviation, \(n = 6\). ***\(P < 0.001\) denotes statistical significance as compared to diabetic control rats in their corresponding week. **\(P < 0.01\) and ***\(P < 0.001\) denotes statistical significance as compared to insulin-treated diabetic rats in their corresponding week.

![Figure 3](image3.png)

**Figure 3:** (a and b) Effect of *Pterocarpus marsupium* on delayed phase of formalin test in diabetic neuropathic pain model. All data expressed as mean ± standard deviation, \(n = 6\). **\(P < 0.01\)** and ***\(P < 0.01\)** denotes statistical significance as compared to diabetic control rats in their corresponding week. *\(P < 0.05\) and **\(P < 0.01\)** as compared to insulin-treated diabetic rats in their corresponding week.
Histopathological Evaluation

The axonal degeneration was evident in the diabetic control group, the morphological picture seen on the pregabalin (10 mg/kg)- and PM (100 and 200 mg/kg)-treated rats when compared to diabetic control rat’s shows decreased areas of degenerated fibers. The axonal degeneration in PM (100 mg/kg)-treated group was found but less than diabetic control. The axonal degeneration was absent in PM (200 mg/kg)-treated rats when compared with diabetic control and also, diabetic control showed more deposition of collagen fibers which was indicative of more fibrosis. In contrast that was absent in pregabalin- and PM-treated animal (Figure 7).

DISCUSSION

In the present study, a significant increase in the blood glucose levels and decrease in nociceptive threshold was observed in rats injected with STZ 40 mg/kg. The results of this study were in correlation to the earlier studies done in STZ-induced rats on similar models of thermal hyperalgesia, mechanical hyperalgesia, thermal allodynia, and formalin-evoked pain.[21,22]
Pregabalin, a selective Ca\textsubscript{2.2} (\(\alpha_{2-\delta}\) subunit) channel antagonist and anticonvulsant, which is successfully being used to treat neuropathic pain syndromes, has reversed the STZ-induced hyperalgesic response in our studies\textsuperscript{[23]} PM, a well-exploited Indian medicinal plant is very much known for its antidiabetic activity\textsuperscript{[24]}. Besides eliciting a strong antidiabetic property, PM was also found to be effective against several other diseases. It is reported to be antiobesity, antihyperlipidemic, anti-inflammatory, anthelmentic, antioxidative, antitumorigenic, and antiulcerative. Treatment with PM extract along with insulin in diabetic rats significantly increased the nociceptive threshold and attenuated allodynia\textsuperscript{[12,25,26]}

In a previous study, STZ-diabetic rats supplemented with methanolic extract of PM for 7 and 14 days showed normalization of streptozotocin-distressed serum glucose by correcting glycosylated hemoglobin, serum protein, insulin, alkaline and acid phosphatase, and albumin levels\textsuperscript{[27]}. A significant reduction in the glucose levels of STZ-induced diabetic rats was previously studied with heartwood of PM signifying its strong antidiabetic properties\textsuperscript{[24]}. In our present study, PM at a dose of 200 mg/kg reduced the glucose level which is in concordance with the previous studies.

Even with rigorous control of blood glucose, prevention of neuropathy is not successful. This might be due to a release of early mediators between the phase of hyperglycemia-induced metabolic changes and nerve cell damage. These mediators are generally called as neuropoietic cytokines such as TNF-\(\alpha\), IL-1\(\beta\), and IL-6\textsuperscript{[28]}. Production of endogenous TNF-\(\alpha\), IL-1\(\beta\), and IL-6 are increased in microvascular and
neural tissues during chronic hyperglycemia, this may cause increased microvascular permeability and nerve damage, thereby initiating and promoting the development of the characteristic lesions of diabetic neuropathy reported that TNF-α and interleukins are important regulators in p38 phosphorylation which leads to cause the pathogenesis of nerve degeneration, suggesting that pain transmission and nerve degeneration are mainly controlled by TNF-α and interleukins.\(^{[29,30]}\) It has also been observed in our study that PM reduced the TNF-α, IL-1β, and IL-6 in diabetic rats, indicating that increased TNF-α, IL-1β, and IL-6 levels are responsible for the decrease in the pain threshold observed in rats.

Experimental studies on neonatal rat STZ model showed a marked decrease in blood glucose and TNF-α when treated with aqueous extract of PM at both doses (100 and 200 mg/kg).\(^{[11]}\) Further, PM extract containing pterostilbene when evaluated for its PGE2-inhibitory activity in LPS-stimulated PBMC showed that it selectively inhibited COX-2.\(^{[31]}\) This suggests the potential use of PM extract in inflammatory disorders and/or inflammatory pain.

The diabetic rats often display typical characteristics of diabetes, that is, polyuria, increased water intake, dehydration, weight loss and muscle wasting and also excessive hair loss and diarrhea. All these manifestations may lead to poor general condition, sometimes to the extent that animals need to be removed from the studies.\(^{[32]}\) Animals in good physical condition might tolerate the testing procedure better than chronically ill animals so that interpretations regarding hyperalgesia and allodynia in the latter might become inaccurate. Hence, maintenance of body weight of experimental rats was ensured throughout this particular study.

In an earlier study, it was recommended to use 245 g of rat for diabetic neuropathy as they showed a negative correlation between body weight and nociceptive threshold and reported that decrease in threshold was mainly caused by the development of a painful neuropathy.\(^{[33]}\) Hence, this study was planned using rats of 240-250 g and the results showed a significant decrease in nociceptive threshold without significant loss of body weight.

The hot plate test involves two types of responses: Paw licking and jumping. Both responses integrate at supraspinal structures with the C and Aδ type I and II sensitive fibers participating in this model.\(^{[34]}\) Here, PM extract showed antinociceptive effect on the diabetic rats in hot plate model which point out its activity through central mechanisms.

Neuropathic pain is often associated with a distinct feature allostynia, which represents an abnormal pain to a stimulus that does not normally provoke pain.\(^{[35]}\) The involvment of small non-myelinated and large myelinated primary nerves (A β and small-diameter nociceptive fibers) in allodynia has been previously accounted.\(^{[36]}\) Recent evidence suggests that diabetic neuropathy leading to allodynia also involves microglia in animal models. The TRPA1 channel has been found obligatory for the existence of a current in a subset of peptidergic sensory neurons and so blockade of TRPA1 would reduce the firing of sensory fibers in response to noxious mechanical stimuli.\(^{[37]}\)

Mechanical alldynia is also linked with central mechanism which is due to peripheral activation of heat-sensor TRPV1 channels that are generally mechanically insensitive. In the dorsal horn of the spinal cord, the increased peripheral input from sensitized primary afferent fibers also results in TRPV1 activation in GABAergic interneuron. TRPV1 opening increases the intracellular calcium concentration which, if prolonged, will result in long-term depression of this inhibitory interneuron.\(^{[38]}\) The GABAergic inhibition exerted on the spinothalamic tract will be released; resulting in enhanced nociceptive inputs to the thalamus. The pre-synaptic TRPV1 activation is an important element in the release of pro-allodynic mediators, and therefore specific antagonists of TRPV1 can certainly block mechanical allodynia. In our study, PM reversed the mechanical allodynia suggestive of its action through either blocking TRPA or TRPV channels.\(^{[39]}\)

The Randall–Selitto test, intended to serve as a tool to assess the effect of analgesic agents on the response thresholds to mechanical pressure stimulation, has been used by a number of researchers to evaluate inflammatory painful responses.\(^{[40-42]}\) The results of Randall–Selitto test performed in this study infer that reduction of mechanical hyperalgesia with the treatment of PM may possibly be due to its anti-inflammatory properties to reduce pain.

The formalin-induced nociception is related with injured tissue. Further, it is believed that this nociception resembles more closely the clinical pain in comparison to other tests that induce pain by mechanical or thermal stimuli.\(^{[35]}\) Formalin test is characterized by two phases: Acute and delayed. The acute phase (0-10 min) is short-lived and initiates immediately after injection and is characterized by C-fiber activation due to peripheral stimuli. The delayed phase (20-40 min) is a longer, persistent period caused by local tissue inflammation, and also by functional changes in the dorsal horn of the spinal cord. Therefore, this phase can be inhibited both by opioids and analgesic agents. Substances that act primarily as central analgesics inhibit both phases while peripherally acting drugs inhibit only the
delayed phase. In the current study, treatment with PM reduced the paw-licking time in both acute and delayed phase implying that PM is involved in both central and peripheral mechanism.

From the morphological study, histological damage in sciatic nerve indicated that diabetes-induced damage in sciatic nerve fibers and axonal degeneration with occasional secondary segmental demyelination. These results are in agreement with the previous studies. Elevated levels of MMP-2 and MMP-9 are known to be involved in the degradation of the extracellular matrix and thickening of basement membrane. This may further lead to nerve injury causing ischemia of nerve tissue and ultimately neural cell death. Pterostilbene which is purified from PM is known inhibitor for MMP-2 to MMP-9 may have resulted in amelioration of affected neurons which is evident from histology. In our study, axonal degeneration was absent in PM (200 mg/kg)-treated rats as compared to diabetic control and also, diabetic control showed more deposition of collagen fibers which was indicative of more fibrosis that was reversed on the administration of PM.

In view of this study results, we conclude that PM attenuated the neuropathic pain in STZ-induced diabetic rats through the modulation of inflammatory cytokine release and correcting the neuronal morphology in diabetic states. These findings suggest that PM may potentially have clinical applications to treat neuropathic pain in diabetic patients.

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REFERENCES


