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Research Article

Neuroprotective Effects of *Lagerstroemia speciosa* L. Extract (Banaba Leaf Extract) in Streptozotocine Induced Painful Diabetic Neuropathy in Laboratory Rats

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Abstract

Background: The present study was design to screen the neuroprotective effects of *Lagerstroemia speciosa* L. on painful diabetic neuropathy. **Materials and Methods:** Diabetes was induced in rats by intraperitoneal injection of single dose of STZ (60 mg kg^{-1}). Neuropathic pain was assessed in diabetic rats by pin prick method, cold allodynia and hot plate method. Pain was developed at 58th day. At the end of experiment animals were scarified and biochemical changes (Lipid peroxidation, reduced glutathione and nitric oxide content) in sciatic nerve were evaluated. Animals were treated with *Lagerstroemia speciosa* L. extract at two doses (50 and 100 mg kg^{-1} p.o.) for 58 days. **Results:** Treatment with *Lagerstroemia speciosa* L. at doses of 50 and 100 mg kg^{-1} significantly restored the reduced body weight and elevated blood sugar level. Further the extract of *Lagerstroemia speciosa* L. showed dose dependent reduction in pain threshold tested by mechanical, cold and thermal hyperalgesia. The level of lipid peroxidation, reduced glutathione and nitric oxide content was significantly prevented. **Conclusion:** The result of present study suggests the antidiabetic, antioxidant and neuroprotectieve property of *Lagerstroemia speciosa* L. in laboratory animals.

Key words: Neuropathic pain, reactive oxygen species, *Lagerstroemia speciosa* L., lipid peroxidation, reduced glutathione

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neuropathy is the most common chronic complication of diabetes and hyperglycemia is the backbone for the pathophysiology of diabetes leading to the development of neuropathic pain. The prevalence of Diabetic Neuropathy (DN) varies from 14-63% depending upon the type of population studied and criteria used to define DN[1]. It is reported that hyperglycemia induces oxidative stress in diabetic neurons, results in the activation of biochemical pathways such as polyol, hexosamine, protein kinase C, advance glycation end products, poly (ADP ribose)polymerase pathway, inflammation and formation of reactive oxygen species[2]. While each pathway may be injurious alone to the cell, collectively they cause an imbalance in the mitochondrial redox state of the cell and lead to excess formation of Reactive Oxygen Species (ROS). Increased oxidative stress leads to activation of the Poly (ADP-ribose) polymerase pathway which regulates the expression of gene involved in promoting inflammatory reaction and neuronal dysfunction to the development of neuropathic pain. There are many agents belonging to different categories have been studied and used for the treatment of DN. The present allopathic treatment of DN is still difficult and there is no single treatment that works for such conditions[3]. The clinical management of DN is often inadequate because of many reasons like inadequate diagnosis, inappropriate drug therapy and complex pathophysiological pathways leading to DN[4].

Lagerstroemia speciosa L. (Lytharceae), leaves contain corosolic acid as an active phytochemicals. It is reported to have wide range of pharmacological activities such as antidiabetic[5], anti-oxidant, anti-inflammatory, anti-hypertensive[6], hepatoprotective, anti-obesity and analgesic[7,8]. These evidences point that *Lagerstroemia speciosa* L. may have potential in diabetic neuropathic pain. As, there is no single evidence available showing the use of *Lagerstroemia speciosa* L. in DN, the present study was design to screen *Lagerstroemia speciosa* L. leaves extract in DN by assessing different behavioral and biochemical parameters.

MATERIALS AND METHODS

Drug and chemicals: Streptozotocin was purchased from Sigma Aldrich Chemie, Germany. Alcoholic Extract of *Lagerstroemia speciosa* L. (AELS) leaves containing 50% corosolic acid was gifted by Kuber Impex Pvt. Ltd., indore, with certificate of analysis. Diagnostic kit for the estimation of glucose was purchased from Span Diagnostic, Mumbai, India. All others chemicals used in the study were of analytical grade and purchased from reputed suppliers.

Experimental animal: Male albino rats of Wistar strain (180-230 g) were used in the entire study. The rats were procured from Lachmi Biotech, Pvt. Ltd., Pune and were placed separately in polypropylene cages (3-4 per cage) with paddy husk as bedding. They were maintained under standard laboratory conditions throughout the experiments. The experimental protocols were reviewed and approved by Institutional Animal Ethic Committee (IAEC) of SSDJ College of Pharmacy, Neminagar, Chandwad (Approval No. SSDJ/ IAEC/ 2012/021).

Induction and assessment of diabetes: Diabetes was induced in the rats by injecting single dose of Streptozotocin (60 mg kg^{-1} i.p.) prepared in chilled citrate buffer (pH 4.4). The control rats received an equal volume of citrate buffer and were used along with diabetic animals. Diabetes was confirmed 48 h after STZ injection. The blood sample was collected via retro-orbital plexus using capillary glass tubes and glucose level was estimated by the enzymatic GOD-POD (Glucose Oxidase Peroxidase) diagnostic kit method. The rats having serum glucose levels more than 250 mg dL^{-1} were considered as diabetic and selected for further study.

Experimental Design for DN: Diabetic rats were divided into different groups (n = 6). Group I served as vehicle control, Group II served as disease control (Diabetic rats, treated with vehicle), Group III rats received AELS (50 mg kg^{-1}, p.o.), Group IV rats received AELS (100 mg kg^{-1}, p.o.) and Group V served as standard group and received Pioglitazone (5 mg kg^{-1}, p.o.). Diabetic rats were tested for the development of neuropathic pain. Rats didn't show any sign of pain for initial 30 days. On day 37 neuropathic pain was started, the animals were then tested for every week and it was found that the rats developed significant pain at 58th days. Treatment with AELS (50 and 100 mg kg^{-1}, p.o.) was continued for 58th days. Threshold of neuropathic pain was checked using different methods. Animals were sacrificed and sciatic nerve was isolate for biochemical study.

Assessment of neuropathic pain

Mechanical hyperalgesia (Pinprick test): The mechanical hyperalgesia was assessed using pin prick test[9]. Rats were individually placed in suspended acrylic chamber on a mesh floor. After the acclimatization period for 30 min, plastic filaments were applied perpendicularly to the planter surface

of both hind paw with sufficient force to bend the plastic filaments; paw withdrawal (lifting) latency was recorded in second. The reaction time was recorded in 0.5 sec units by a stopwatch. The cut off time of the paw withdrawal was 15 sec. A withdrawal time of more than 6 sec therefore is regarded as a positive response[9]. The paw withdrawal time was measured weekly after confirmation of diabetes.

Cold allodynia: The method was suggested by various authors. In that they dip the hind paw gently in ice cold water and observed the paw withdrawal latency[10]. In the present study we modified the method and used acetone to produce cold allodynia. In this the cotton swab was deeped into fixed volume of acetone, it was then applied perpendicularly to the planter surface of both hindpaw for measurement of the paw withdrawal (lifting) latency, The cut-off time was 15 sec.

Thermal hyperalgesia: In this test, rats were individually placed on a hot plate (Eddy's Hot-plate appratus or chid, Nashik) with the temperature adjusted to 55±1° C. The latency to the first sign of paw licking or jump responce to avoid the heat was taken as an index of the pain threshold, the cut-off time was 15 sec in order to avoid damage to the paw[11].

Assessment of biochemical parameters: At the end of study the animals were euthanasiously sacrificed and sciatic nerve was quickly isolated and transferred into ice-cold Tris hydrochloric buffered saline (pH 7.4). It was weighed on electronic balance WENSAR, (Model-PGB200). The Sciatic nerve was cross-chopped with surgical scalpel into fine slices. The tissues were then minced and homogenised in chilled Tris hydrochloride buffer (10 mM, pH 7.4) to a concentration of 10% w/v. The homogenate was centrifuged at 10,000 rpm at 0° C for 15 min using high speed cooling centrifuge (Remi C-24). The clear supernatant was used for the estimation of lipid peroxidation[12] and reduced glutathione level[13]. Nitric oxide was estimated calorimetrically using Griess reagent[14].

Statistical analysis: All the values are expressed as mean±S.E.M. Statistical significance between more than two groups was tested using one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test as appropriate using computer based fitting program (Prism, Graphpad). Differences were considered to be statistical significant when $p<0.05$.

RESULTS

Effect of AELS on serum glucose level and body weight: Blood glucose level and body weight of all the group was monitored after 30 days of STZ injection. Diabetic control rats showed a significant ($p<0.001$) rise in serum glucose level and decline in body weight as compared to normal control rats. Treatment with AELS (50 and 100 mg kg^{-1}, p.o.) showed significant ($p<0.001$) decreased in blood glucose level and improvement in body weight compared to control rats (Table 1).

Effect of AELS on diabetic neuropathy induced mechanical hyperalgesia: The paw withdrawal latency in diabetic control rats before the induction of neuropathic pain was not significantly changed as compared to normal control rats. A significant ($p<0.001$) decreased in mean paw withdrawal latency was observed in the diabetic rats after 4 weeks of STZ injection as compare to normal control rats. Rats treated with AELS (50 and 100 mg kg^{-1}, p.o.) and Pioglitazone (5 mg kg^{-1}, p.o.) for 8 weeks significantly ($p<0.001$, $p<0.01$) and dose dependently increased the change in mean paw withdrawal latency as compare to diabetic control rats (Fig. 1).

Effect of AELS on diabetic neuropathy induced cold allodynia: In cold allodynia the paw withdrawal latency was significantly ($p<0.001$) decreased in diabetic rats after 37 days of STZ injection as compare to normal control rats. Rats treated with AELS, 50 and 100 mg kg^{-1} for 8 weeks significantly ($p<0.001$, $p<0.05$) and dose dependently increased the change in mean paw withdrawal latency as compare to diabetic control rats (Fig. 2).

Effect of AELS on thermal hyperalgesia: Hot plate test was used to study thermal hyperalgesia. The diabetic rats showed significant ($p<0.001$) reduction in mean paw withdrawal latency after 4 weeks of STZ injection as compare to normal control rats indicating that the diabetic rats had sensation for

Table 1: Effect of AELS on serum glucose level and body weight in diabetic rats

Groups	Serum glucose (mg dL^{-1})	Body weight (g)
Normal control	110.30±6.19	248±10.48
STZ disease Control	290.00±15.42***	174±13.53*
STZ+AELS (50)	106.50±10.51###	205±08.09
STZ+AELS (100)	138.20±12.36###	230±15.83##
STZ+Pio (5)	098.72±12.58###	239±11.14###

All values are presented as Mean±SEM, (n=6), $p<0.01$**, $p<0.001$*** compared to control group, $p<0.01$##, $p<0.001$### compared to disease group

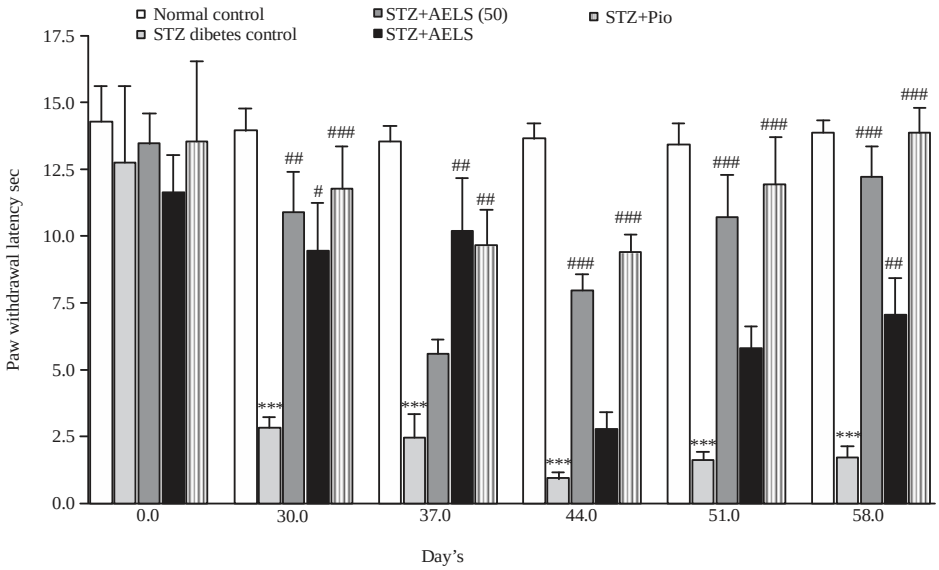

Fig. 1: Effect of AELS (50, 100 mg kg^{-1}, p.o.) on Mechanical hyperalgesia in diabetic neuropathic pain. All values are presented as Mean±SEM, (n = 6), p<0.05*, p<0.01**, p<0.001*** compared to control group, p<0.05$^{\#}$, p<0.01$^{\#\#}$, p<0.001$^{\#\#\#}$ compared to disease group

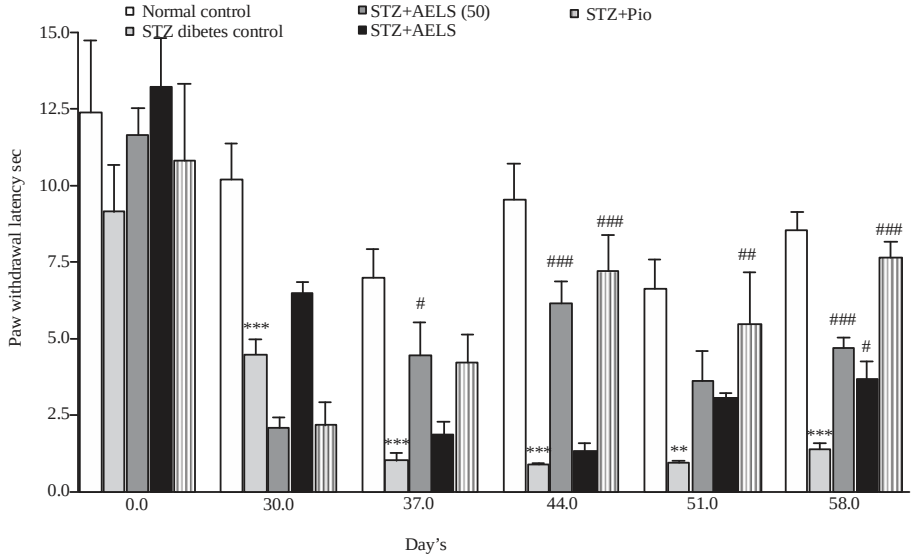

Fig. 2: Effect of AELS (50, 100 mg kg^{-1}, p.o.) on Cold allodynia in diabetic neuropathic pain. All values are presented as Mean±SEM, (n = 6), p<0.05*, p<0.01**, p<0.001*** compared to control group, p<0.05$^{\#}$, p<0.01$^{\#\#}$, p<0.001$^{\#\#\#}$ compared to disease group

heat. Treatment of diabetic rats with AELS (50, 100 mg kg^{-1}) and Pioglitazone 5 mg kg^{-1} for 8 weeks significantly (p<0.01, p<0.001) and dose dependently increased the change in mean paw withdrawal latency as compare to diabetic control rats (Fig. 3).

Effect of AELS on markers of oxidative stress: Markers of oxidative stress such as lipid peroxidation, reduced glutathione and nitric oxide content were studied in sciatic nerve preparation from all the groups. It was found that in diabetes rat the level of lipid peroxidation and nitric oxide content was significantly increased and reduced glutathione level was significantly decreased as compare to control rats. Chronic treatment with AELS (50 and 100 mg kg^{-1}. p.o.) for 58 days showed significant (p<0.05) decreased in lipid per oxidation level and nitric oxide

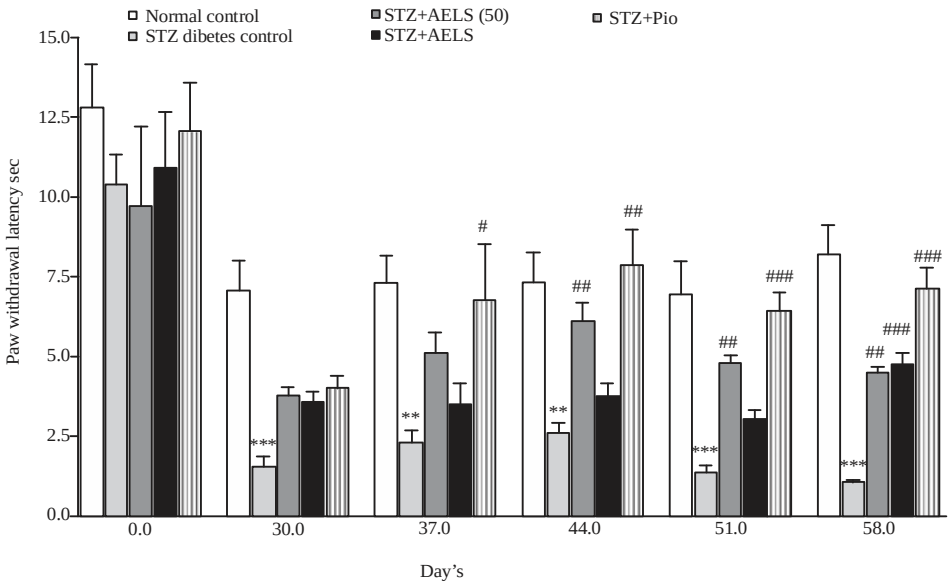

Fig. 3: Effect of AELS (50, 100mg kg^{-1}, p.o.) on Thermal hyperalgesia (Hot plate method) in diabetic neuropathic pain. All values are presented as Mean±SEM, (n = 6), p<0.05*, p<0.01**, p<0.001*** compared to control group, p<0.05$^{\#}$, p<0.01$^{\#\#}$, p<0.001$^{\#\#\#}$ compared to disease group

Table 2: Effect of AELS on markers of oxidative stress in diabetic neuropathy

Groups	Lipid Peroxidation (MDA/g of tissue)	Reduced Glutathione (µg/g of tissue)	Nitric Oxide (nmole/g of tissue)
Normal control	0.918±0.157	1.461±0.064	10.290±0.969
STZ disease Control	4.320±0.537*	0.477±0.072**	24.580±1.621***
STZ+AELS (50)	1.273±1.062$^{\#}$	1.355±0.129$^{\#\#}$	11.490±0.646$^{\#\#\#}$
STZ+AELS (100)	1.334±0.154$^{\#}$	1.921±1.152$^{\#}$	14.970±2.218$^{\#\#\#}$
STZ+Pio (5)	1.204±0.119$^{\#}$	1.538±0.067$^{\#\#}$	10.36±0.707$^{\#\#\#}$

All values are presented as Mean±SEM, (n = 6), p<0.05*, p<0.01**, p<0.001*** compared to control group, p<0.05$^{\#}$, p<0.01$^{\#\#}$, p<0.001$^{\#\#\#}$ compared to disease group

content as compared to diabetes rats whereas significant increased in reduced glutathione level compared to diabetic control rats (Table 2).

DISCUSSION

The present study provides valuable information about therapeutic potential of AELS in DN. In the present study, diabetes was induced by administration of single dose of STZ (60 mg kg^{-1}, i.p.)[15]. The STZ damages the DNA of pancreatic β cells and thereby triggers multiple biochemical pathways such as polyol pathway, hexosamine pathway, Protein Kinase C pathway (PKC), Advance Glycation End (AGE) product and Poly Adipose Ribose Polymerase (PARP), pathway all of these pathways contribute in the production of oxidative stress by generating ROS in mitochondrial redox state cell which results in nerve damage and neuropathy[16]. In the present study diabetic rats showed significant elevation of serum glucose level and reduction on body weight compared to control rats. Elevation in blood glucose level might be due to STZ induced pancreatic damage and reduction in body weight might be due to increased muscle wasting and loss of tissue proteins which is in line with previous studies[15,17]. The AELS treatment reveres the changes in body weight and blood glucose level which suggest the ant diabetic activity of AELS[18].

The DN is associated with decrease in paw withdrawal latency and which can be evaluated by behavioral nociceptive test like mechanical hyperalgesia, cold allodynia and thermal hyperalgesia. A change in nociception is well reported in earlier studies of diabetic neuropathic pain[9,11,15,17]. In the present study, mechanical hyperalgesia is evaluated using pin prick method, cold allodynia using acetone and thermal hyperalgesia response assessed by using hot plate method. Assessment of behavioral responses to external stimuli in animals provides valuable information regarding the mechanisms of abnormal sensation and pain associated with diabetes[19]. It has been reported that unmyelinated C fibers are responsible for pain and A-delta fibers for carrying temperature, crude touch and pricking pain sensations[20]. In the present study, both hot and cold sensations were altered

in diabetic rats which indicate damage of the respective fibers and development of neuropathic pain. Treatment with AELS significantly attenuated the response of pain in diabetic neuropathic condition. It might be due to tight control of blood glucose level, decreased the overproduction of reactive oxygen species which regulates the expression of gene involved in promoting inflammatory reaction and neuronal dysfunction in neuropathic pain[16].

Reactive oxygen species are critically involved in the development and maintenance of neuropathic pain 16. Present study showed a significant rise in lipid peroxidation (LPO), Nitric Oxide (NO) level and decreases in the level of reduced glutathione (GSH) in sciatic nerve which indicates the involvement of oxidative stress in DN. Increases the level of LPO and NO during inflammation may be damage to axons. Both combines with superoxide to form peroxinitrite which causes protein nitration or nitrosylation, lipid peroxidation, DNA damage and cell death also produced toxic effects on the nerve tissue leading to neuropathic pain. Total nitric oxide, an indicator of nitrosative stress, is increased in the experimental model of diabetic neuropathy[11]. Chronic treatment with AELS in DN showed the attenuation of increased lipid peroxidation and nitric oxide levels whereas decreased GSH level which might be due to its NOS inhibitory potential as well as potent free radical scavenging activity of AELS. Earlier study reported that *Lagerstroemia speciosa* possesses anti-diabetic[18], anti-oxidant, anti-obesity[7] and hepato-protective activity. It mainly contains corosolic acid as an active phytoconstituent which is reported to possess anti-diabetic, anti-oxidant[21], anti-inflammatory, anti-hypertension and analgesic activity[6]. Corosolic acid reduces blood glucose level by activating the transport of glucose across the cell membranes, Insulin-Mimetic (peptide analogs) activity[22], GLUT4 activation, α-amylase and α-glucosidase inhibitory activity[18].

In conclusion, chronic treatment of AELS in diabetes neuropathic pain significantly reversed mechanical and thermal hyperalgesia, allodynia and algesia in rat. AELS treatment significantly and dose dependently reduces the development of oxidative stress during pain by scavenging free radicals. These neuroprotective effects of AELS might be due to its strong antidiabetic and antioxidant activity. This is the first report of AELS which showed protective effect in DN.

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