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Protective effect of *Lagerstroemia speciosa* leaves extract against streptozotocin induced diabetic neuropathy in experimental rats

Sameer Nasikkar, Prashant Mali, Preeti Bavage and Jayesh Jain

Abstract
Objective: The present study was designed to evaluate effects of *Lagerstroemia Speciosa* leaves extract in streptozotocin induced diabetic neuropathy.
Methods: Diabetes was induced in Wistar rats by injecting streptozotocin (60 mg/kg/intraperitoneally). Four week after the confirmation of diabetes, diabetic rats were treated with *Lagerstroemia Speciosa* Leaves (LS) extract (200 and 400 mg/kg, p.o) for next four weeks. Rats were subjected to evaluate biochemical, behavioural, and oxidative stress parameters. Hyperalgesia and cold allodynia test were performed on 7th, 14th, 21th and 28th day in all groups to assess the extent of neuropathy by Eddy's hot plate and tail immersion test respectively. Further, the walking function test to assess motor response and histopathology of sciatic nerve were performed at the end of 4th week treatment.
Results: Treatment with LS extract (200 and 400 mg/kg) significantly reduced elevated blood glucose level, and lipid profiles and restored the reduced body weight. Further, 4 week treatment of LS significantly attenuated hyperalgesia, cold allodynia. Walking function in diabetic treated rats were improved as compared to diabetic control rats. Elevated levels of malondialdehyde, and nitric oxide and decreased glutathione levels and superoxide dismutase were restored significantly after 4 week LS treatment. Consequently, LS extract (200 and 400 mg/kg) treated rats showed regeneration of neuronal fibres of sciatic nerve in dose dependent manner.
Conclusion: *Lagerstroemia Speciosa* leaves extract has shown amelioration in diabetic neuropathic symptoms which may be attributed by its antihyperglycemic and antioxidant effect.

Keywords: Diabetic neuropathy, *Lagerstroemia Speciosa*, Diabetes mellitus, Sciatic nerve

1. Introduction

Diabetes mellitus is one of the most common metabolic disorder which is a leading cause of morbidity and mortality in world's growing population. Diabetes is a chronic condition characterised by hyperglycemia resulting from lacking of insulin secretion (type 1), resistance to insulin action (type 2) or both. Long term hyperglycemia leads to tissue injury due to development of diabetic neuropathy (DN) associated with type 1 and type 2 diabetes [1]. Hyperglycemia and oxidative stress are plays key role in the pathogenesis of the neuropathies. Persisting hyperglycemia provokes oxidative stress due to an exhibition of biosynthetic pathways including hexosamine biosynthesis, activation of sorbitol pathway, activation of poly ADP-ribose polymerase [2], impaired insulin/C peptide action, and formation of advanced glycation end product (AGE) [3]. These mechanisms do not work in isolation, but strongly interact in a mutually facilitator fashion. Nitrosative stress and induction of the enzyme poly (ADP-ribose) polymerase form one important link between physiological stressors such as reactive oxygen species (ROS) and the pro-inflammatory mechanisms [4]. Nitrosative stress and oxidative stress, leads to axonal degeneration, demyelination, and atrophy [5, 6]. Various cytokines and excitatory neurotransmitters also contribute to down regulation of pain threshold of the neurons [7].
At present, the available synthetic drugs for the treatment of diabetic complications includes antioxidants selective serotonin reuptake inhibitors, antidepressants [8], anti-arrhythmics, polyphenols, anticonvulsants and opioids, which has met limited success in clinical trials [9-11]. A large number of plants used in the traditional medicine have now become a part of the modern world health care system as they show promising therapeutic effect, minimal side

Effects, cheap and easily available. Various medicinal plants/plant extracts containing flavonoids, alkaloids, phenolic compounds, terpenoids, saponins and phytosterol type chemical constituents were found to be effective in the management of diabetic complications. This effect might be attributed to amelioration of persistent hyperglycemia, oxidative stress and modulation of various metabolic pathways involved in the pathogenesis of diabetic complications [12].

Lagerstroemia speciosa also called Banaba is a tropical plant found in many parts of Southeast Asia including the Philippines, Vietnam, Malaysia and southern China. Several studies indicates the beneficial role for Lagerstroemia speciosa (especially, corosolic acid) in terms of antidiabetic [13], antiobesity [14], hypoglycemic [15], anti-inflammatory and free radicle scavenging activity [16]. Besides, no adverse effects have been observed or reported in animal studies or controlled human clinical trials [17]. In continuation of these reported activities, the present study was designed to investigate the effect of *Lagerstroemia speciosa* leaves extract on streptozotocin induced diabetic neuropathy.

2. Material and methods

2.1 Animal

Male Wistar rats (180- 220 g) were procured from National Institute of Biosciences, Pune. Rats were placed separately in polypropylene cages (six per cage) randomly with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 20 ^{0}C, relative humidity 55 ± 10% and 12 hrs light and 12 hrs dark cycles throughout all the experiments. Animals had free access of water and standard laboratory feed ad libitum (Nutrivet Lab., Pune) prior to the dietary manipulation. The experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee SCOP/IAEC/2014-15/186. The animals were shifted to the laboratory one hour prior to the experiment.

2.2 Drugs and Chemicals

Lagerstroemia Speciosa leaves extract (Banaba leaves extract 1% Corosolic Acid) were purchased from Kuber Impex Ltd. Indore. Streptozotocin was purchased from Sigma-Aldrich. Biochemical estimation kits (prietest) were purchased from Robnic India Pvt Ltd Mumbai. Other chemicals were analytical grade purchased from local suppliers Pune, India.

2.3 Preparation of drug solutions

Lagerstroemia Speciosa leaves (LS) extract was suspended in 2% gum acacia solution prepared in distilled water and STZ was dissolved in cold citrate buffer (pH 4.4). Drugs solutions were freshly prepared.

2.4 Experimental induction of diabetes and assessment

Streptozotocin (STZ) was dissolved in 0.1 M sodium citrate buffer, pH 4.4 and administered at the dose of 60 mg/kg through i.p. route. STZ-treated rats received 5% of glucose solution instead of water for 24 h after injection of STZ in order to reduce death due to hypoglycemic shock [18]. Blood samples were taken from the tail vein 48 h after STZ or vehicle injection to measure blood glucose levels. Only animals with fasting blood glucose levels over ≥ 250 mg/dl were considered diabetic and used for the further study.

2.5 Treatment schedule

Four week after diabetic induction, diabetic rats and age matched normal rats were randomly divided into four groups (n= 6): Normal control (NC)**,** Diabetic control, Diabetic + LS (200 mg/kg), and Diabetic + LS (400 mg/kg). LS was daily administered by oral gavage for next four weeks (7th, 14th, 21th and 28th day). Normal control and diabetic control rats received 2% acacia alone. At the end of 28th day blood glucose level and body weight were measured.

2.6 Sample collection

For the determination of blood glucose, tail was suspended in warm water and swiped with spirit. The tail vein was punctured with sharp needle to ooze out drop of blood and blood glucose level were measured by commercial glucose estimation strips. For biochemical estimations, blood was withdrawn from retro-orbital plexus of overnight fasted rats using a micro-capillary technique under light anaesthesia.

2.7 Estimation of biochemical parameters

Serum estimation for total cholesterol [19], triglyceride [20] and total protein [21] was estimated using the commercial diagnostic kits and semi auto-analyser.

2.8 Behavioural parameters

2.8.1 Assessment of thermal hyperalgesia

Hyperalgesia is an early symptom of neuropathic pain and diabetic neuropathy pain in experimental animals. Four week post STZ injection change in pain threshold were assessed before and after every week of four week treatment schedule in all four groups (NC, DC, DC+LS 200 mg/kg and 400 mg/kg) by Eddy's hot plate method. Paw withdrawal latency (cut off time: 15sec) of each rat was determined using hot plate which is maintained at constant temperature of 55 ºC [22].

2.8.2 Assessment of cold allodynia

Cold allodynia were assessed before treatment and after every treatment week with LS, with tail immersion test. The tail flick latency of each rat was determined by immersing the tail into the cup filled water that had a constant temperature of 10 ºC and recording the tail withdrawal latency (cut of time: 20 sec) manually with stop watch. A shortened duration of immersion indicates allodynia [23].

2.8.3 Assessment of walking function

Walking function test was performed to assess the motor functioning in rats. The device used for the walking test was a rod of 6 cm diameter and 1 m long, maintained horizontally 40 cm above a table. The rod was graduated in order to allow the measurement of the distance covered by the animals. Three trials per session were performed. For each trial (60 sec maximum), each rat was placed at an extremity of the rod, and the time needed to walk 1 m distance was recorded. If the animal falls down or is unable to walk the 1 m distance, 60 sec were credited. For each animal, the mean duration of the three trials was calculated and retained as the characteristic value [24].

2.9 Estimation of oxidative stress parameters

Estimation of oxidative stress was performed in supernatant of sciatic nerve homogenate. a) Estimation of lipid peroxidation was done by measuring the levels of

malondialdehyde (MDA) by the method of Mihara *et al* [25] and expressed as nmol MDA per mg of protein. b) Reduced glutathione (GSH) levels were estimated by the method of Ellman as described previously [26]. c) Superoxide dismustase activity was estimated by the method of Kono [27]. d) Nitric oxide (NO) concentration was calculated using a standard curve for sodium nitrite and expressed as ng/mg of protein [28].

2.10 Histopathological examination

After termination of experiment, sciatic nerves from all groups of rats were subjected to histopathological studies. Sciatic nerves removed from the rats of each group under study were preserved in 10% neutral buffered formalin for 72 hours. The nerves were rinsed in running water for removal of preservative. The tissue was further processed in ascending grades of alcohol for dehydration and was cleared in xylene and finally embedded in paraffin block. The tissue was then sectioned on automated microtome and about 5 µ sections were stained with haematoxylin and eosin protocol. The stained slides were evaluated by qualified veterinary pathologist.

2.11 Statistical analysis

The results were expressed as mean ± S.E.M, n=6. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests and two-way ANOVA followed by Bonferroni post-test using Graph Pad Prism 5.03 software. $P \leq 0.05$ were considered significant.

3. Results

3.1 Effect of LS extract on blood glucose levels.

A single intraperitoneal injection of STZ (60 mg/kg body weight) showed significant increase in blood glucose level in diabetic control rats when compared with normal control rats (P<0.001). Diabetic rats treated with LS (200 and 400 mg/kg body weight) for four weeks significantly decreased the elevated blood glucose level when compared with diabetic control rats (P<0.001), whereas LS (400 mg/kg) has shown significant decrease in blood glucose level as compared to low dose of LS (200 mg/kg) (P<0.001).

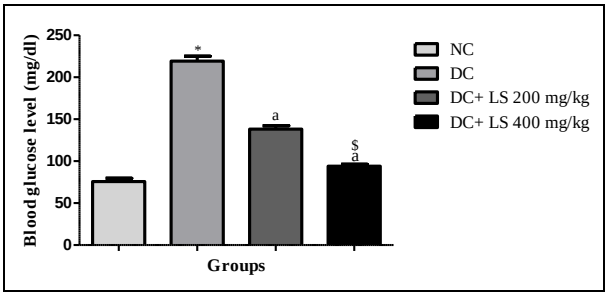

Fig 1: Effect of LS extract on blood glucose levels.

n= 6, all values are represented as mean ± SEM. Analysed by One way ANOVA, followed by Tukey's test. $^{*}P$ <0.001 compared to Normal Control group. ^{a}P <0.001 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.

NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.2 Effect of LS extract on body weight

After 10 weeks of study significant decreased in body weight of diabetic control rats was observed when compared to normal control rats(P<0.05). Four weeks treatment of LS (200 and 400 mg/kg body weight) in diabetic rats was found slight increase in body weight when compared to diabetic control rats (P<0.01, P<0.01 respectively).

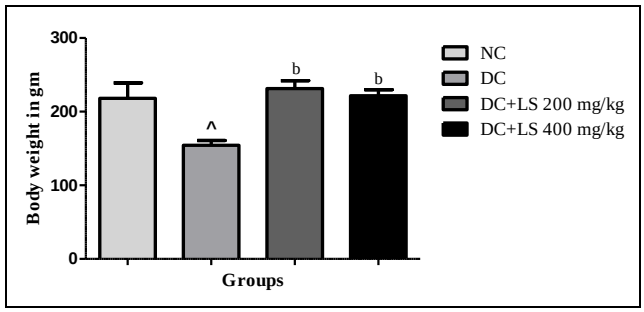

Fig 2: Effect of LS extract on body weight.

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. $^{\wedge}P$ < 0.05 compared to Normal Control group. ^{b}P< 0.01 compared to Diabetic Control group.

NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.3 Effect of LS extract on serum total cholesterol (TC) levels.

A single intraperitoneal injection of STZ in diabetic control rats were observed with significant rise in the serum total cholesterol levels when compared to normal control rats (P<0.001). Four weeks of LS treatments (200 and 400 mg/kg body weight) in diabetic rats significantly reduced serum total cholesterol levels (P<0.001, P<0.001 respectively), whereas LS (400 mg/kg) has shown significant reduction cholesterol level as compared to low dose of LS (200 mg/kg) (P<0.001).

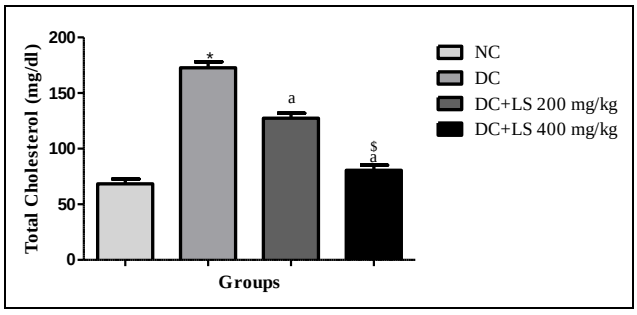

Fig 3: Effect of LS extract on total cholesterol levels.

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.

NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.4 Effect of LS extract on serum triglyceride (TG) levels

Diabetic control rats were observed with significant rise in the serum triglyceride levels when compared to normal control rats (P<0.001). Four weeks of LS treatments (200 and 400 mg/kg body weight) in diabetic rats significantly restored serum triglyceride levels (P<0.01, P<0.001 respectively).

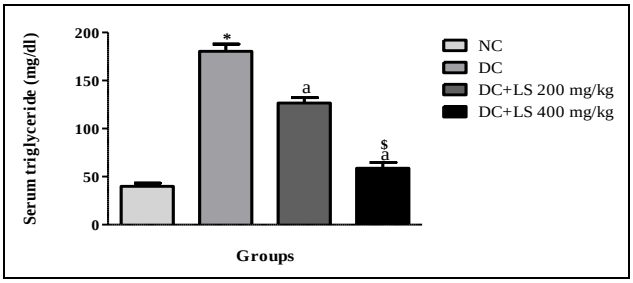

Fig 4: Effect of LS extract on serum triglyceride levels

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.5 Effect of LS extract total proteins levels

Diabetic control rats were observed with significant decrease in the total proteins levels when compared to normal control rats (P<0.001). Four weeks of LS treatments (200 and 400 mg/kg body weight) in diabetic rats significantly restored the levels of total proteins (P<0.01, P<0.001 respectively)

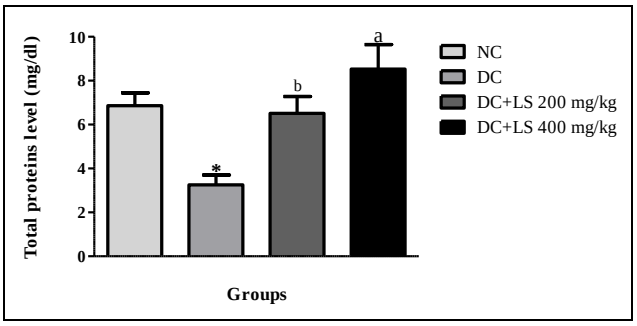

Fig 5: Effect of LS extract total proteins levels

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P < 0.01 compared to Diabetic Control group.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.6 Effect of LS extract on paw withdrawal latency

Repeated measure two-way ANOVA revealed that there was significant influence of LS extract treatment on diabetic neuropathy induced hyperalgesia (P<0.001), at the end of treatment. Analysis further revealed time dependent changes. The Bonferroni's post hoc test revealed that diabetic rats showed significant reduction in paw withdrawal latency on 7th, 14th, 21th and 28th day indicating that diabetic rats had thermal hyperalgesia. Further preventive treatment with LS (200 and 400 mg/kg) significantly reduced thermal hyperalgesia on 21th (P<0.001), 28th day (P<0.001).

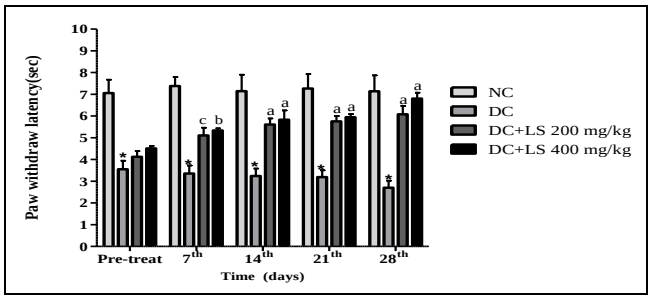

Fig 6: Effect of LS extract on paw withdrawal latency

n= 6, all values are represented as mean ± SEM. Analyzed by two-way ANOVA followed by Bonferroni post-test. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P < 0.01, ^{c}P<0.05 compared to Diabetic Control group.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.7 Effect of LS extract on tail flick latency

Repeated measure two-way ANOVA revealed that there was significant influence of LS extract treatment on diabetic neuropathy induced cold allodynia (P<0.001), at the end of treatment. Analysis further revealed time dependent changes. The Bonferroni's post hoc test revealed that diabetic rats showed significant reduction in tail flick latency on 7th, 14th, 21th and 28th day indicating that diabetic rats had cold allodynia. Further, treatment with LS (200 and 400 mg/kg) significantly increased the tail flick latency on 21th (P<0.01, P<0.001, respectively) and 28th day (P<0.001).

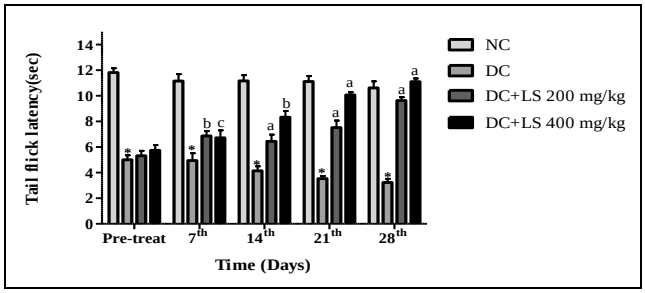

Fig 7: Effect of LS extract on tail flick latency

n= 6, all values are represented as mean ± SEM. Analyzed by two-way ANOVA followed by Bonferroni post-test. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P < 0.01 and ^{c}P<0.05 compared to Diabetic Control group.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.8 Effect of LS extract on walking function test

Diabetic control rats were observed with significant increase in duration for travelling 1 meter distance when compared to normal control rats (P<0.001). Four weeks of LS treatments (200 and 400 mg/kg body weight) in diabetic rats showed significant improvement in shortening of time (P<0.01, P<0.001 respectively) as compared to diabetic control. Whereas LS (400 mg/kg) has shown significant improvement in shortening of time than low dose LS (200 mg/kg) (P<0.001).

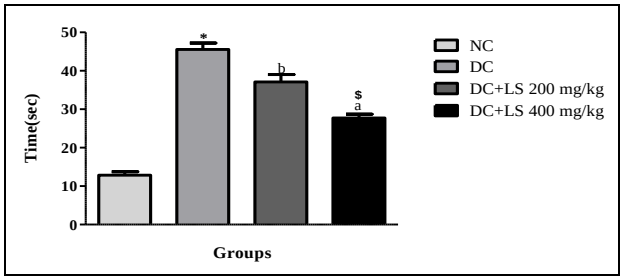

Fig 8: Effect of LS extract on walking function test

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P<0.01 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.9 Effect of LS extract on oxidative stress markers.

Oxidative stress generates with chronic hyperglycemia which leads to free radical production and lipid peroxidation and this is evaluated by the level of oxidative markers such as Malondialdehyde (MDA), Glutathione (GSH), Superoxide dismutase (SOD), Nitric oxide (NO) etc. Diabetic control group shows significant (P< 0.001) increase in MDA, (P<0.001) in NO level whereas a significant decrease (P<0.001) in GSH and SOD level were observed. 4 week treatment of LS (200 and 400 mg/kg body weight) shows significant changes (P<0.001) in the oxidative markers compared to normal control group. Hence LS extract was found to have antioxidant activity on sciatic nerve homogenate.

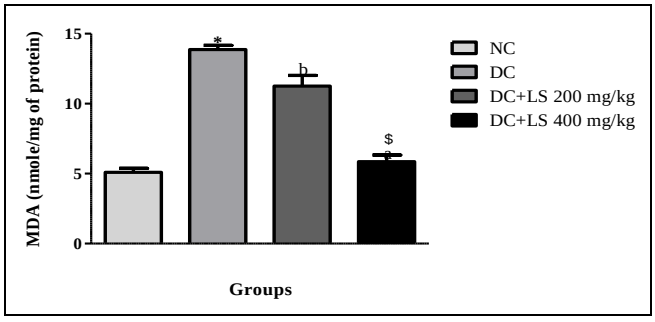

Fig 9: Effect of LS extract on MDA levels

n= 6, all values are represented as mean ± SEM. $^{*}P$ < 0.001 compared to Normal Control group. Analyzed by one-way ANOVA followed by Tukey's multiple comparison. ^{a}P <0.001, ^{b}P<0.01 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

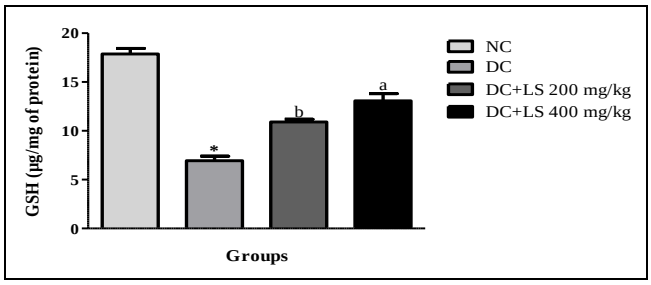

Fig 10: Effect of LS extract on GSH levels

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P<0.01 compared to Diabetic Control group
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

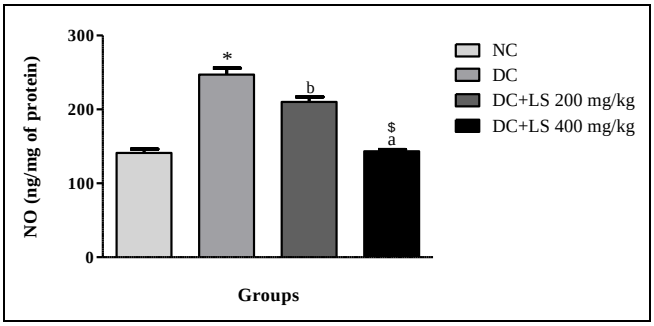

Fig 11: Effect of LS extract on NO levels

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P$ < 0.001 compared to Normal Control group. ^{a}P <0.001, ^{b}P<0.01 compared to Diabetic Control group and $^{\$}P$ < 0.001 compared to LS 200 mg/kg.
NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

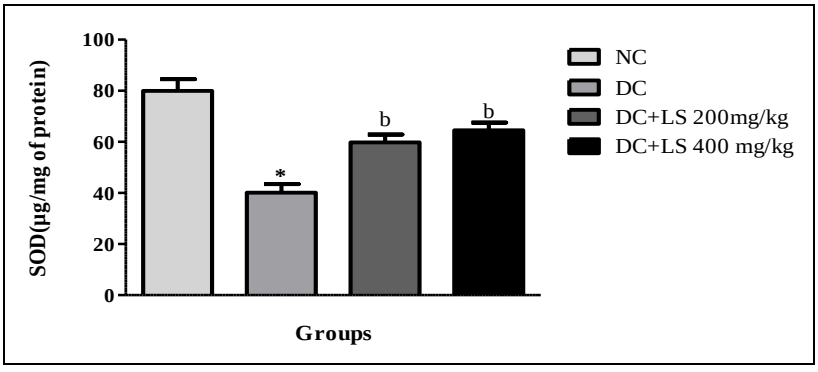

Fig 12: Effect of LS extract on SOD activity

n= 6, all values are represented as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. $^{*}P < 0.001$ compared to Normal Control group. $^{b}P < 0.01$, compared to Diabetic Control group

NC= Normal control, DC= Diabetic control, LS= *Lagerstroemia Speciosa* leaves extract.

3.10 Histopathology of Sciatic nerve

The H&E stained lateral section of diabetic control sciatic nerve (B) showed focal degenerative changes, break in continuity of nerve fibres and vacuolar changes compared to normal nerve (A). Four weeks treatment of LS (200 mg/kg & 400 mg/kg body weight) in diabetic rats was shown to improve the structural integrity in neuronal fibres and vacuoles in dose dependent manner.

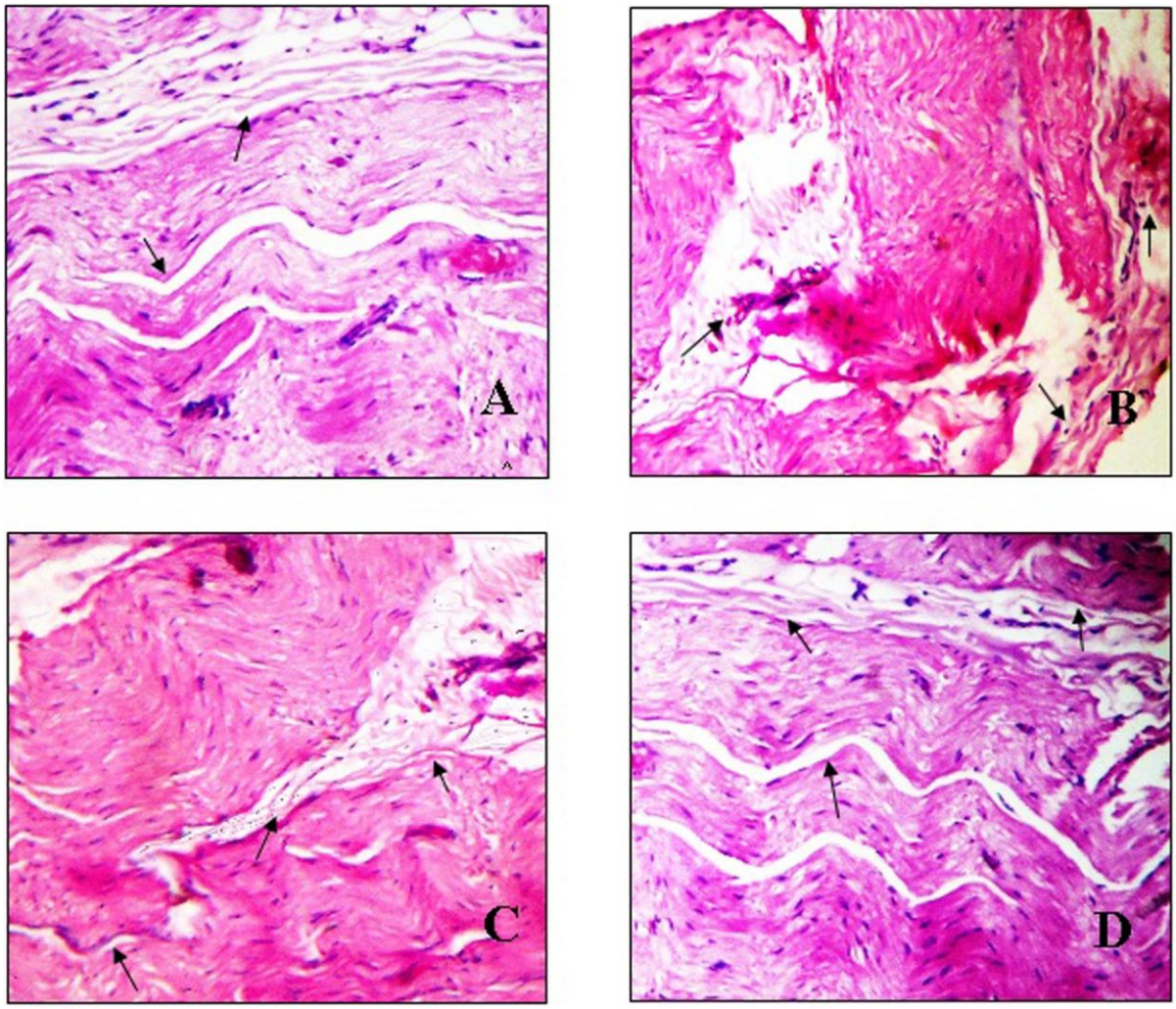

Fig 13: Photomicrograph of H&E stained lateral sections of sciatic nerves A. Normal nerve (black arrows point out intact nerve fibres), B. Diabetic nerve (black arrows point out neuronal degeneration), C. LS 200 mg/kg treated diabetic nerve (black arrows point out regeneration of neuronal fibres damaged due to oxidative stress), D. LS 400 mg/kg treated diabetic nerve (black arrows point out prominent regeneration of nuclear neuronal fibres damaged due to oxidative stress).

4. Discussion

Diabetic peripheral neuropathy (DPN) is allay with substantial morbidity, mortality and reduced quality of life and affects up to 50% of people with diabetes [29].

Diabetic peripheral neuropathy (DPN) is characterised by pain numbness and tingling in the extremities and slow motor nerve conduction. DPN occurs due to chronic untreated hyperglycemia. Not only neurodegenerative changes are precipitate but repair mechanism also affects in persisting hyperglycemic environment. Reduced levels of neurotrophins including nerve growth factor and insulin-like growth factor have been noticed in experimental diabetes [30]. Clinical symptoms of DPN are allodynia, hyperalgesia due to elevated nociceptive response, decreased motor nerve conduction velocity, neuronal hypoxia, reduced pain threshold, etc. [31]. Similar symptoms are exhibited by STZ induced diabetic animals [32]. STZ injected rats model exhibits clinicopathological features including biochemical, oxidative and metabolic alterations which also conferred in human [33]. STZ impaired the DNA of pancreatic β cells and triggers multiple pathways like, activation of protein kinase-C with consequent formation of ROS and AGE resulting in neuronal impairment and neuropathy [34]. Intraperitoneal administration of STZ (60 mg/kg) can produce significant elevation in blood glucose level. Further, STZ-induced diabetic rats showed reduction in body weight due to increased muscle wasting and loss of tissue proteins which is in accord with previous reports [20, 35]. In the present investigation, rise in blood glucose level and loss in body weight were halted in LS treated rats when compared with vehicle treated diabetic rats. Abnormalities in lipid profile like hypercholesterolemia and hypertriglyceridemia are link with important risk factors of diabetes mellitus found in 40% of diabetic cases. Increased triglyceride concentration

combine with increased low density (LDL) concentration constitute an especially high risk of coronary heart disease (CHD). This is a common finding with diabetic patient's results from accumulation of LDL either by excess production or decrease catabolism or both. Increased level of serum triglyceride and cholesterol were found in STZ induced diabetic rats in our study. Treatment with LS (200 mg/kg and 400 mg/kg) significantly controls the elevated lipid levels of diabetic rats compared to diabetic control rats. This finding suggest that treatment with LS can potentially ameliorate lipid abnormalities in STZ induced diabetes in experimental rats.

Protein has a minimal effect on blood glucose level with adequate insulin. However, with insulin deficiency, gluconeogenesis proceeds rapidly and contribute to an elevated blood glucose level [36]. This process forms glucose from storage protein, thus protein level decline in diabetes. LS (200 and 400 mg/kg) significantly increased serum protein level in experimental diabetic rats.

Eddy's hot plate, tail flick and walking function test have been reported method for evaluation of behavioural parameters such as peripheral hyperalgesia, cold allodynia and sensorimotor deficit respectively in laboratory animals [22-26]. Intraperitoneal administration of STZ results into worsening of sensory-motor nerve fibres resulting decrease in withdrawal latency time in diabetic rats. Significant dose dependent amelioration were seen on 21th and 28th day in reduction of thermal hyperalgesia and increased tail flick latency compared to control group.

Walking function test is a non-invasive method of assessing the functional status of the sciatic nerve during the regeneration process, because proper walking requires coordinated function involving sensory input, motor response, and cortical integration [26]. Diabetic rats showed significantly increased latency time to travel 1 m distance in walking function test, which was significantly improved after the LS (200 and 400 mg/kg) treatment.

Chronic persisting hyperglycemia instigates biosynthetic pathways leads to increase in oxidative stress which contribute to development and progression of diabetic complication. Pain threshold of the neurons is reduced due to ROS such as super oxide dismutase, hydroxyl radical, and peroxynitrite which impair blood supply to the neurons leading to defective neuronal function and hypoxia. These conditions were studied by assessing the biochemical markers like SOD, GSH, MDA, and NO.

SOD and MDA are endogenous enzymes closely in twined with oxidative stress [37]. Superoxide and hydroxyl free radicals are mainly responsible for vascular endothelial damage [38]. Superoxide anions are also believed to cause increase in aldose reductase and protein kinase C activity which are further implicated in pain perception [39]. SOD provides antioxidant protection from superoxide anions by transforming them to H_2O_2. MDA is elevated in stress conditions. It is mainly responsible for destruction of lipid membrane via rearrangement of the double bond in the unsaturated fatty acids of the membrane caused tissue damage [40]. For the conversion of glucose into sorbitol nicotinic acid adenine di-nucleotide phosphate (NADPH) is used. NADPH is an important co factor necessary to regenerate reduced glutathione (GSH) and GSH is a scavenger of reactive oxygen species (ROS). Depletion of GSH could exacerbate intra cellular oxidative stress and there by contribute to diabetic complications [41]. In the present study treatment with LS shows antioxidant effect by increasing SOD activity and GSH level.

The formation of peroxynitrite from superoxide and NO appears to be an important step, introducing an element of nitrosative stress into the aetiology of diabetic neuropathy [42]. In the experimental model of DPN, total NO, an indicator of nitrosative stress, is increased [43]. LS treatment (200 and 400 mg/kg) attenuates the increased NO level.

Further, these ROS responsible for demyelination of nerve fibres, results in low sensory-motor nerve conduction and decrease nociceptive threshold. Histopathology investigation of sciatic nerve revealed that LS treated diabetic rats had lesser injuries to nerves compared with diabetic control. Consequently, LS (200 and 400 mg/kg) treated rats shows regeneration of neuronal fibres in dose dependent manner. Here, the data demonstrating the neuronal degeneration due to oxidative stress and undergoes apoptosis in chronic hyperglycemic state. In the present study LS treatment significantly reduced neuronal damage by lowering blood glucose level, attenuating the increased level of ROS, restoration of depleted GSH and SOD.

5. Conclusion

The present data conclude that the repeated dose treatment of *Lagerstroemia Speciosa* leaves extract for four weeks in diabetic rats not only attenuate the hyperglycemic condition but also reversed DPN symptoms. It is a potent anti-diabetic and antioxidant, which has observed as its ameliorative effects.

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7. Competing of interest

The authors declare that they have no competing interests.

8. References

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