Protective effects of Ethanolic extract of *Alpinia calcarata* Roscoe rhizome on streptozotocin-induced diabetic peripheral neuropathy in rats

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Abstract

Background: Diabetic peripheral neuropathy (DPN) is a common and debilitating complication of diabetes, leading to nerve damage and impaired pain sensation. **Objectives:** The present study aimed to investigate the protective effects of ethanolic extract of *Alpinia calcarata* Roscoe rhizome (EEACR) on rats' streptozotocin (STZ)-induced DPN. **Materials and Methods:** Male Wistar rats were divided into control, STZ-induced diabetic, and two treatment groups (250 and 500 mg/kg EEACR). Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg), and the treatment groups received EEACR orally for four weeks. **Results:** The results demonstrated a significant reduction in blood glucose levels and increased body weight in the EEACR-treated groups compared to the STZ-induced diabetic group. Moreover, EEACR treatment significantly ameliorated mechanical allodynia, thermal hyperalgesia, and cold allodynia in a dose-dependent manner. Biochemical analysis showed that EEACR treatment significantly reduced oxidative stress and increased antioxidant enzyme activities, such as superoxide dismutase, catalase, and glutathione peroxidase, in the sciatic nerves of treated rats. Histopathological evaluation revealed reduced nerve damage and degeneration in the EEACR-treated groups and improved myelin sheath and axonal integrity. **Conclusion:** This study provides evidence that the ethanolic extract of *Alpinia calcarata* Roscoe rhizome possesses protective effects against streptozotocin-induced diabetic peripheral neuropathy in rats, potentially

through its antioxidant properties and ability to reduce oxidative stress. The findings suggest that EEACR may be a promising therapeutic agent for managing diabetic peripheral neuropathy.

Key-words: Diabetic, peripheral neuropathy, oxidative stress, *Alpinia calcarata*

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Olokoba et al., 2012). The global prevalence of diabetes is rapidly increasing, affecting millions of people worldwide (Kharroubi and Darwish, 2015). The International Diabetes Federation (IDF) estimates that by 2045, the number of people with diabetes will reach 784 million, a 51% increase from the 2021 statistics (Christ, 2020). Diabetes can lead to various complications, including retinopathy, nephropathy, and neuropathy, with diabetic peripheral neuropathy (DPN) being the most common and debilitating complication. Diabetic peripheral neuropathy affects approximately 50% of diabetic patients, causing significant morbidity and reduced quality of life (Yang et al., 2022; Selvarajah et al., 2019; Sloan et al., 2021). DPN is characterized by pain, numbness, and tingling in the extremities, particularly the feet (Røikjer and Ejskjaer, 2022). The pathogenesis of DPN is multifactorial, with factors like oxidative stress, inflammation, and advanced glycation end-products playing crucial roles in the development and progression of the condition (Vinik et al., 2013). Current therapeutic options for DPN, including analgesics and anticonvulsants, primarily focus on alleviating symptoms rather than treating the underlying pathophysiological mechanisms. Therefore, there is a pressing need for novel therapeutic interventions to prevent or reverse DPN's progression.

Natural products derived from medicinal plants have been used for centuries to treat various ailments, including diabetes and its complications. *Alpinia calcarata* Roscoe, a plant from the Zingiberaceae family, is widely used in traditional medicine systems in South and Southeast Asia to treat a range of conditions, such as inflammation, pain, and respiratory ailments (Rahman and Islam MS, 2015; Wijayasiriwardena and Premakumara, 2012; Kong et al., 2000). The rhizome of *A. calcarata* is rich in bioactive compounds, including flavonoids, terpenoids, and phenolic compounds, which exhibit potent antioxidant, anti-inflammatory, and analgesic properties (Arawwawala et al., 2012; Arambewela et al., 2010; Arambewela et al., 2004). These properties have

garnered interest in the potential therapeutic effects of *A. calcarata* for managing diabetes and its complications.

Ethanolic extracts have demonstrated significant biological activities due to their ability to solubilize many phytochemicals. The ethanolic extract of *A. calcarata* rhizome has been shown to possess potent antioxidant and anti-inflammatory activities, which may contribute to its potential protective effects against diabetes and associated complications. Furthermore, previous studies have reported the hypoglycemic and antihyperlipidemic effects of *A. calcarata* rhizome extract in streptozotocin (STZ)-induced diabetic rats, suggesting its potential role in managing diabetes (Rajasekar et al., 2014).

Although the ethanolic extract of *A. calcarata* rhizome has been shown to exhibit hypoglycemic and antihyperlipidemic effects in STZ-induced diabetic rats, its potential protective effects against DPN remain unexplored. This study, therefore, aims to investigate the protective effects of ethanolic extract of *A. calcarata* rhizome on STZ-induced peripheral neuropathy in rats.

Materials and Methods

Chemicals

STZ was acquired from Sigma Aldrich, India. While diagnostic kits for biochemical evaluations were procured from Krishgen Biosystem, India. All other chemicals utilized in the study were of analytical grade.

Plant material and its extraction

Alpinia calcarata Roscoe rhizomes were gathered from IIHR (Indian Institute of Horticulture and Research), Bengaluru. The botanical specimens were verified and confirmed by the same.

Freshly harvested *Alpinia calcarata* Roscoe rhizomes were sectioned into small fragments and subjected to air-drying for 12 to 14 days under shaded conditions. Subsequently, 500 grams of the desiccated rhizome powder were subjected to extraction with 1.5 liters of ethanol via a Soxhlet extraction apparatus for 4 hours. Following filtration of the resultant solution, the filtrate was concentrated under reduced pressure at 55 °C, yielding an 18.5% (w/w, based on dry weight) extract preserved at 4 °C for future utilization. A co-precipitate containing polyvinylpyrrolidone (PVP; molecular weight 44,000) was synthesized by blending the

crude ethanolic extract (concentration: 1.0 mg/mL in ethanol) with PVP in a 1:1 (w/w) ratio (Arambewela et al., 2004).

Animals

The investigation was conducted using male Wister albino rats weighing 180 to 230 grams; rats were maintained in a controlled environment (temperature: 22 ± 2°C, humidity: 75 ± 5%, 12-hour light/dark cycle) within the research facility for the duration of the study. The animals were given a nutritional diet and water *ad libitum*. Before the commencement of the experiment, the rats were given a one-week acclimation period to adjust to their new surroundings. The study's experimental protocol received approval from the Institutional Animal Ethics Committee, KLE University's College of Pharmacy, Bengaluru (03/HP/2021), which was established in line with the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India.

Induction of diabetic neuropathy and treatment

Diabetes was induced through a single intraperitoneal injection of streptozotocin (STZ) at 55 mg/kg, dissolved in ice-cold 0.1 M citrate buffer (pH 4.4), as previously described (Bhatt and Veeranjaneyulu, 2010; Kamdi et al., 2021). Forty-eight hours after STZ administration, plasma glucose levels were assessed to confirm diabetes induction. Animals exhibiting plasma glucose concentrations exceeding 250 mg/dl were identified as diabetic and included in subsequent analyses. Diabetic animals were classified according to their plasma glucose concentrations and body weights.

Group I – Normal Control

Group II – Positive control (STZ; 55mg/kg b.w, i.p)

Group III – STZ + *Alpinia calcarata* Roscoe rhizome extract (250mg/kg) for 4 weeks

Group IV – STZ + *Alpinia calcarata* Roscoe rhizome extract (500mg/kg) for 4 weeks

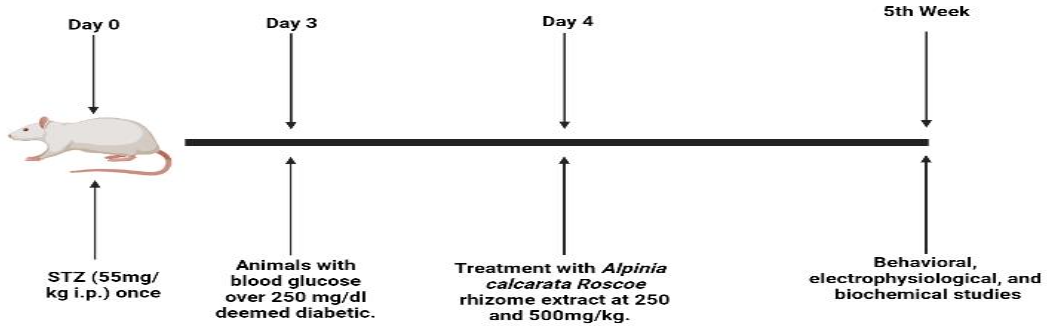

Figure 1: Diagram illustrating experiment layout

Evaluation parameters

Body weight and plasma glucose levels

In the Streptozotocin (STZ)-induced diabetes peripheral neuropathy model, body weight was measured at the end of the study. Blood specimens were obtained from the retro-orbital sinus of the rats under light anesthesia upon completion of the experimental procedures. Subsequently, the blood samples underwent centrifugation to isolate serum. Plasma glucose levels were quantified using specialized enzyme-linked immunosorbent assay (ELISA) kits.

Behavioral parameters

Thermal Hyperalgesia

The rat tail was submerged in a heated water bath maintained at 46°C. Observations were made for indications of discomfort or reflexive retraction of the tail, and the latency period was documented using a stopwatch. A maximum duration of 12 seconds was established as the threshold. Decreases in the time taken for tail retraction indicate heightened pain sensitivity. After recording each response, the tail was meticulously dried after each response (Padgaonkar et al., 2017).

Cold Hyperalgesia

The rat tail was dipped in a container filled with cold water (4ºC). A rapid retraction or tail movement in response to the low temperature was observed and documented, with a maximum observation period of 10 seconds. A decrease in the time taken for the tail to retract indicates the presence of cold hyperalgesia (Ostovar et al., 2019).

Rota-Rod Performance

The Rota-rod assessment is a widely employed method for examining motor coordination in rats, specifically by measuring the capacity of rats to maintain their position on a rotating rod with a 75 mm diameter. The rotation speed was adjusted to enable normal rats to remain on the rod for 5 minutes. Rats underwent three practice sessions, each spaced 3 to 4 hours apart. During the evaluation, the rat was positioned on the rod, which rotates at 15 and 25 rpm speeds. The duration each rat successfully stayed on the Rota rod was documented (Vera et al., 2013).

Grip Strength

A grip strength measurement was used to assess the grip strength of rat models in this study. Prior to the experimentation, the animals underwent an acclimatization process, during which they were placed on the apparatus for a designated period. To perform the measurement, each rat was suspended by its tail, ensuring a nearly horizontal orientation above the grid of the grip strength meter. Subsequently, a gentle force was applied to the base of the tail, aligning with the axis of the sensor until the animal released its hold on the grid. The exerted force by the rat was documented in either kilograms or newtons units (Nayak et al., 2021).

Electrophysiological parameter

Measurement of Nerve conduction velocity

The assessment of nerve conduction velocity was conducted non-invasively utilizing a PowerLab data acquisition system. An anesthetic combination of ketamine and xylazine (ranging from 80-100 mg/kg and 5-10 mg/kg, respectively, administered intraperitoneally) was used during the experimental procedure. The sciatic nerve was stimulated by applying supramaximal electrical stimuli through an electrode operating at 8V and a frequency of 20 Hz. Compound muscle action potential latencies were determined using bipolar electrodes. Motor nerve conduction velocity (MNCV) can be calculated by subtracting the proximal latency from the distal latency and dividing the resulting value by the distance separating the stimulation and recording electrodes (Addepalli and Suryavanshi, 2018).

$$\text{Nerve conduction velocity (m/sec)} = \frac{\text{Distance between stimulation sites}}{\text{Latency of M wave (sciatic notch) - Latency of M wave (Achilles tendon)}}$$

Oxidative stress parameters

At the end of the experiment, sciatic nerves were isolated and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) using a probe homogenizer. The homogenate was divided into aliquots to obtain post-mitochondrial and post-nuclear fractions (Garud and Kulkarni, 2018). The protein concentration in the tissue homogenate was determined using a previously described method (Lowry et al., 1951). Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) (Ohkawa et al.,

1979). The tissue sulfhydryl groups were assessed by determining the levels of reduced glutathione (GSH) (Ellman, 1959). Catalase activity was determined by measuring the amount of hydrogen peroxide decomposed per minute (Aebi, 1984). Superoxide dismutase (SOD) activity was measured using a previously described method (Paoletti et al., 1986).

Assay of cytokines

The TNF-α and IL-6 were measured using ELISA and according to the method adopted by Whiteside (Meager, 2006) using the kits following the manufacturer's instructions (Endogen Company, USA).

Histopathology

After collecting blood samples, the sciatic nerves were carefully isolated and submerged in a 10% formalin solution for fixation. Subsequently, the fixed nerves were immersed in paraffin wax in preparation for sectioning. Utilizing a microtome, ultra-thin sections of 5 micrometers in thickness were obtained. For histological evaluation, these sections undergo staining with Mayer's hematoxylin and eosin for general morphology, Masson's trichrome to visualize collagen distribution, and the Kulchitsky-Pal to assess myelin integrity.

Statistical analysis

All the data were analyzed by one-way ANOVA following post hoc Dunnett's multiple comparisons using Graph pad Prism software (version 9).

Results

Impact of *Alpinia calcarata* Roscoe Rhizomes Administration on Body Weight, and Blood Glucose

The diabetic group experienced a notable decline in body weight, mitigated by administering *Alpinia calcarata* at 250mg/kg and 500mg/kg compared to the diabetic group (Figure 2). Elevated blood glucose concentrations were observed in diabetic rats relative to healthy controls (317.4mg/dl versus 105.8mg/dl, $P<0.001$). A 4-week *Alpinia calcarata* treatment regimen led to a substantial decrease in blood glucose concentrations at dosages of 250mg/kg (278.7mg/dl, $P<0.05$) and 500mg/kg (254.8 mg/dl, $P<0.001$) compared to the diabetic group.

Impact of *Alpinia calcarata* Roscoe rhizomes treatment on behavior parameters

The observed decrease in response latency and tail withdrawal latency was markedly significant (P<0.001) in the diabetic group compared to the normal group for both the tail immersion test in hot and cold water. This reduction in latency was considerably counteracted by a four-week *Alpinia calcarata* treatment at all evaluated dose levels when compared with the diabetic group. The assessment of the Rota-rod response was carried out using the Rota-rod apparatus. Compared to normal animals, diabetic rats substantially declined motor coordination. Their capacity to sustain themselves on the rotarod apparatus was significantly diminished (P<0.001) compared to non-diabetic control animals. Administration of 250mg/kg and 500mg/kg *Alpinia calcarata* in diabetic animals led to an increase in retention time. Nerve strength was assessed by measuring grip strength in diabetic rats. Diabetic animals significantly reduced grip strength compared to normal rats, indicating muscle weakness. Administration of *Alpinia calcarata* for 4 weeks significantly improved grip strength latency compared to STZ-treated rats (Figure 3).

Impact of *Alpinia calcarata* treatment on NCV

Diabetic rats exhibited a substantial decline in nerve conduction velocity (NCV) compared to their healthy counterparts (P<0.001). A 4-week regimen of *Alpinia calcarata* treatment led to a notable enhancement in NCV among diabetic rats when administered at doses of 250mg/kg (P<0.001) and 500 mg/kg (P< 0.001) in comparison to the diabetic control group (Figure 4).

The impact of *Alpinia calcarata* therapy on oxidative stress markers

In diabetic animals, a marked elevation in oxidative stress within the sciatic nerves was observed, as evidenced by a considerable increase in malondialdehyde (MDA) levels and a decrease in glutathione (GSH), superoxide dismutase (SOD), and catalase concentrations when compared to non-diabetic animals. *Alpinia calcarata* treatment at 250mg/kg and 500mg/kg led to a substantial decrease in MDA levels (P< 0.001 and P<0.001, respectively) compared to the diabetic group. Additionally, GSH levels experienced a significant rise following *Alpinia calcarata* administration at doses of 250mg/kg (P<0.05) and 500mg/kg (P<0.01). Furthermore, catalase and SOD concentrations showed a notable increase after *Alpinia calcarata* treatment compared to the diabetic control group (Figure 5).

IL-6 and TNF-α Levels

As shown in Figure 6, Levels of cytokines TNF- α and IL-6 in rats after induction of diabetic with STZ revealed high significant increase with percent change (85% and 80%), respectively, compared with the control group. A 4-week regimen of *Alpinia calcarata* treatment led to a notable enhancement in both TNF- α and IL-6 among diabetic rats when administered at doses of 250mg/kg ($P<0.001$) and 500 mg/kg ($P< 0.001$) in comparison to the diabetic control group.

Effect of *Alpinia calcarata* treatment on histopathology of sciatic nerves

In the *Alpinia calcarata*-treated group, H&E staining revealed a well-preserved nerve morphology with intact myelin sheaths and minimal infiltration of inflammatory cells compared to the disease group. The disease group displayed evidence of degenerative changes, including axonal swelling, loss of myelin, and infiltration of inflammatory cells. These findings suggest a protective effect of *Alpinia calcarata* treatment on the sciatic nerves (Figure 7).

Masson's trichrome staining demonstrated a more uniform and well-organized collagen distribution in the *Alpinia calcarata*-treated group compared to the disease group. The disease group exhibited increased deposition of disorganized collagen fibers around the nerve, indicating fibrosis. This result suggests that *Alpinia calcarata* treatment may help prevent fibrotic changes in the sciatic nerves (Figure 8).

Kulchitsky-Pal staining revealed a lower incidence of neurofibrillary tangles in the *Alpinia calcarata*-treated group compared to the disease group. The disease group displayed more neurofibrillary tangles, suggesting a possible neurodegenerative process. The reduced neurofibrillary tangles in the treated group indicate that *Alpinia calcarata* may have a neuroprotective effect on the sciatic nerves (Figure 9).

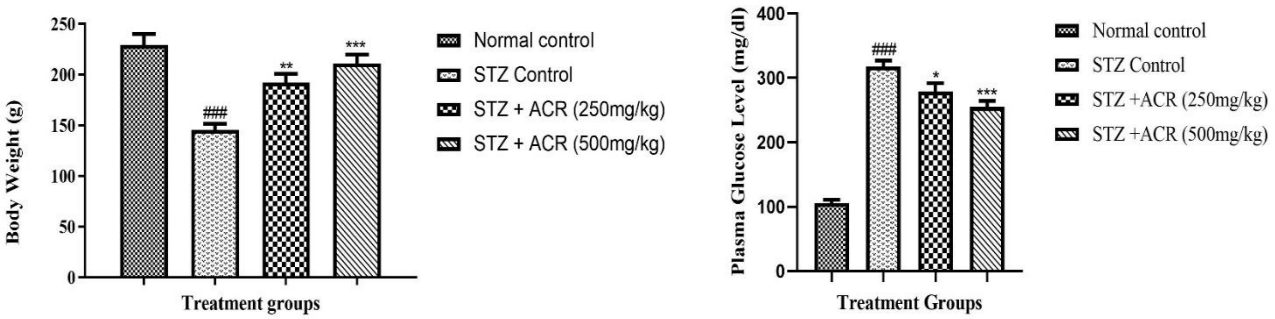

Fig. 2. Effect of treatment *Alpinia calcarata* on body weight and plasma glucose levels. All data are expressed as Mean ± SEM (n=6). ### P<0.001 when compared with normal control, ***P<0.001, **P<0.01, *P<0.05 when compared with diabetic control.

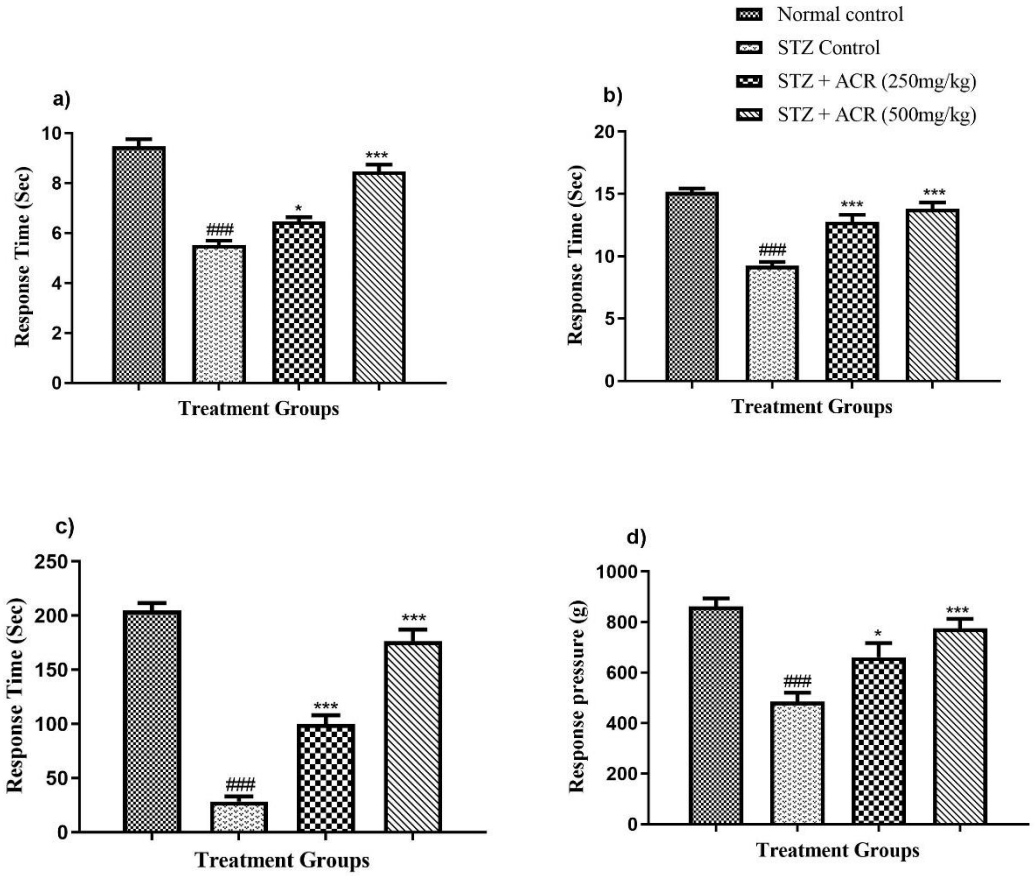

Fig. 3. Effect of *Alpinia calcarata* treatment on a) Tail immersion in hot water, b) Tail immersion in cold water, c) Rota-rod test, and d) Grip strength test. All data are expressed as Mean ± SEM (n = 6). ### P<0.001 when compared with normal control, *P<0.05, **P<0.01, ***P<0.001 when compared with diabetic control.

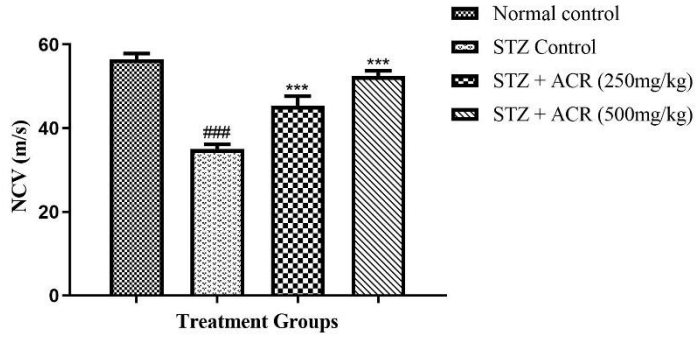

Fig. 4. Effect of *Alpinia calcarata* treatment on NCV. All data are expressed as Mean ± SEM (n = 6). ### P<0.001 when compared with normal control, ***P<0.001 when compared with diabetic control.

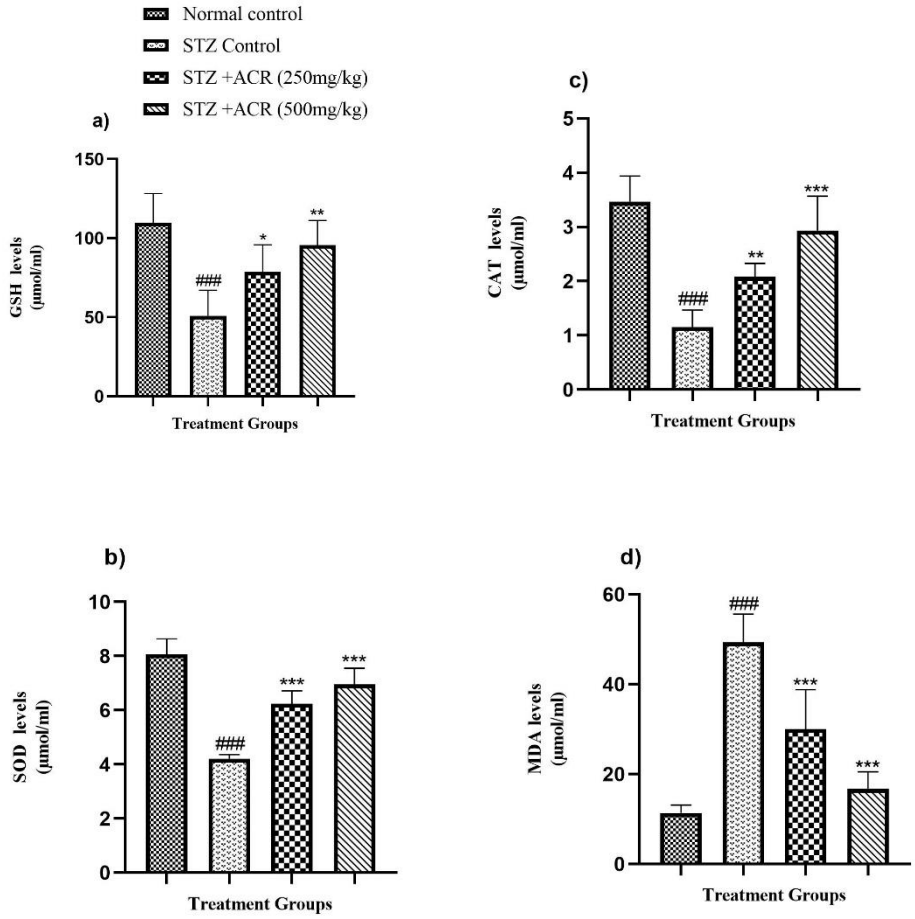

Fig. 5. Effect of *Alpinia calcarata* treatment on a) GSH, b) CAT, c) SOD, and d) MDA. All data are expressed as Mean ± SEM (n = 6). ### P<0.001 when compared with normal control, *P<0.05, **P<0.01, ***P<0.001 when compared with diabetic control.

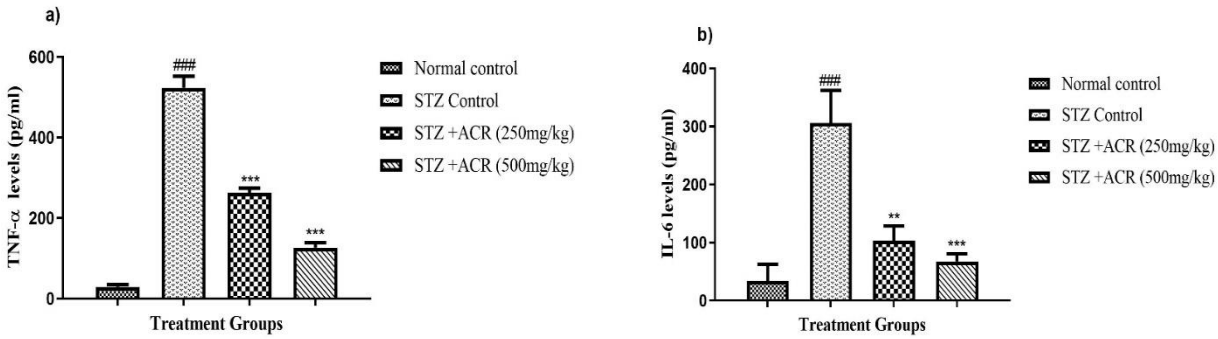

Fig. 6. Effect of *Alpinia calcarata* treatment on a) TNF-α, b) IL-6. All data are expressed as Mean ± SEM (n = 6). ### P<0.001 when compared with normal control, **P<0.01, ***P<0.001 when compared with diabetic control.

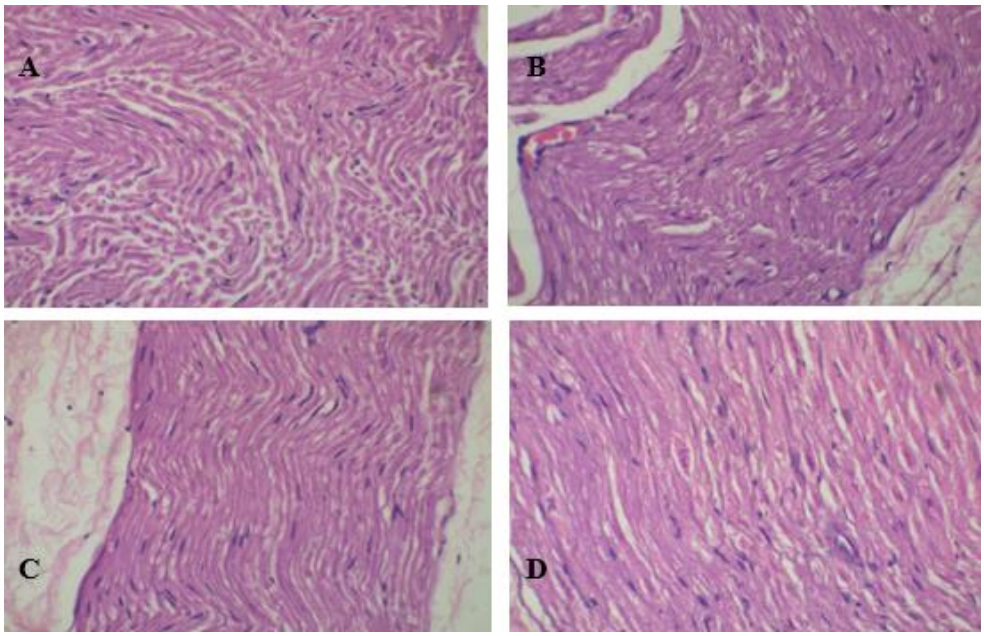

Fig. 7. Effect of *Alpinia calcarata* treatment on sciatic nerves- H&E staining (40×) a) Normal Control: showing a well-preserved nerve morphology with intact myelin sheaths and minimal infiltration of inflammatory cells b) Diabetic Control: showing degenerative changes, including axonal swelling and loss of myelin c,d) *Alpinia calcarata* (250 mg/kg and 500 mg/kg): showing normal histology, myelin sheath minimal infiltration of inflammatory cell.

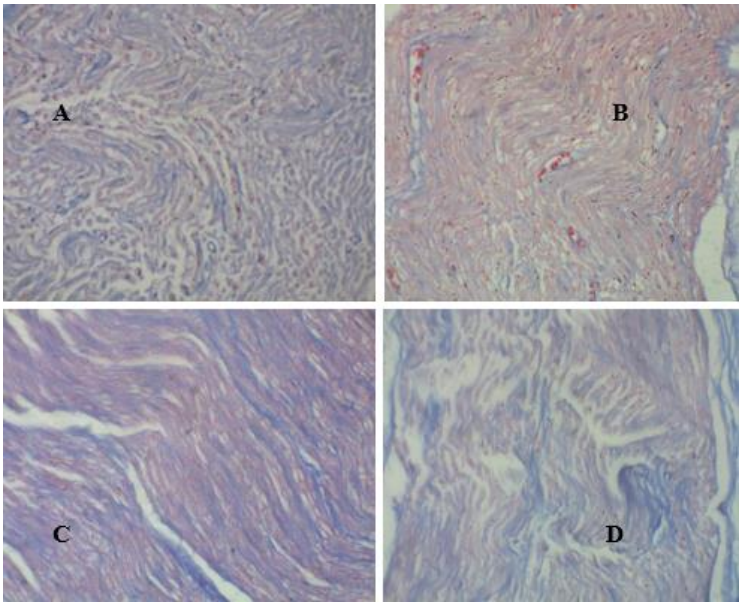

Fig. 8. Effect of *Alpinia calcarata* treatment on sciatic nerves- Masson's trichrome staining (40×) a) Normal Control: showing lower incidence of neurofibrillary tangles b) Diabetic Control: displayed more neurofibrillary tangles, suggesting a possible neurodegenerative process. c,d) *Alpinia calcarata* (250 mg/kg and 500 mg/kg): revealed a lower incidence of neurofibrillary tangles.

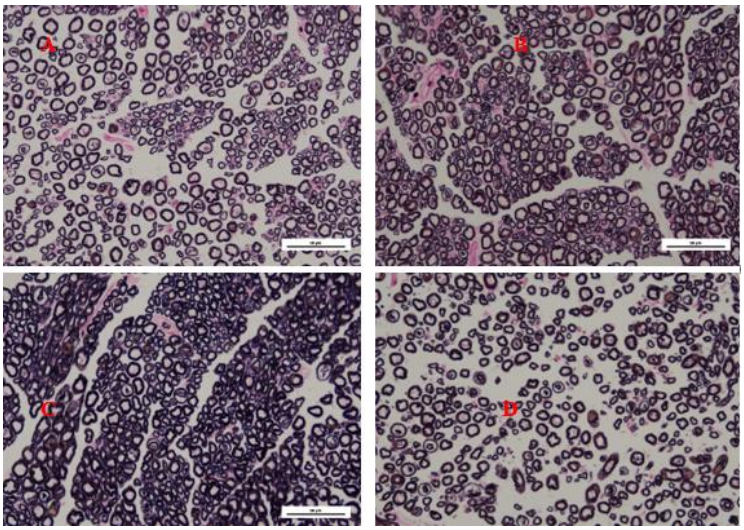

Fig. 9. Effect of *Alpinia calcarata* treatment on sciatic nerves- Kulchitsky-Pal staining (40×) a) Normal Control: showing uniform and well-organized collagen distribution b) Diabetic Control: showing increased deposition of disorganized collagen fibers around the nerve, indicating fibrosis. c,d) *Alpinia calcarata* (250 mg/kg and 500 mg/kg): showing normal histology and uniform and well-organized collagen distribution.

Discussion

Diabetic complications, including neuropathy, nephropathy, retinopathy, and cardiomyopathy, are primarily attributed to uncontrolled blood sugar levels, elevated advanced glycation end products (AGEs), and increased receptors for AGEs (RAGE), leading to stress-induced inflammatory cascades. Diabetic peripheral neuropathy (DPN) is a persistent microvascular complication characterized by decreased vibration perception and nerve conduction velocity (Yagihashi et al., 2007). Although the precise mechanism underlying DPN remains elusive, it is predominantly associated with heightened oxidative stress and increased polyol pathway activity (Ahmad and Hoda, 2020). Various risk factors contribute to peripheral nerve damage, such as glycated hemoglobin (HbA1c) and collagen accumulation in peripheral nerves (Sugimoto et al., 1997). This accumulation provokes neuronal injury by activating apoptotic and inflammatory cascades by stimulating inflammatory and apoptotic mediators, including nuclear factor-kappa B (NF-kB) and tumor necrosis factor-alpha (TNF-α). Concurrently, an elevated polyol flux leads to sorbitol and reactive oxygen species (ROS) buildup, impairing nerve conduction velocities (Suryavanshi and Kulkarni, 2017; Padgaonkar et al., 2017).

The overexpression of NF-kB is central to neuronal injury, as it activates inflammatory mediators like tumor growth factor-beta (TGF-ß), TNF-α, and interleukin-6 (IL-6). Additionally, the arachidonic acid pathway is triggered in peripheral nerves, and cyclooxygenase-2 (COX-2) levels rise in response to NF-kB activation (Khan et al., 2013). This complex interplay of factors contributes to the development and progression of diabetic peripheral neuropathy, emphasizing the need for further research to elucidate the underlying mechanisms and develop effective treatments.

In individuals with diabetes, either inadequate insulin production or heightened insulin resistance disrupts the movement of glucose across cellular membranes, resulting in elevated blood glucose levels. Because of this impaired glucose transport, stored fats are metabolized for energy, which can lead to significant weight loss. This phenomenon is particularly evident in patients with type 1 diabetes. *Alpinia calcarata* has been demonstrated to promote insulin secretion, facilitating glucose transport across cell membranes and decreasing plasma glucose levels (Wang et al., 2009). The observed decrease in plasma glucose levels following *Alpinia calcarata* administration may be attributed to its insulin-stimulating properties. Furthermore, *Alpinia calcarata* treatment has been linked to a notable increase in overall body weight. Uncontrolled

diabetes can lead to neuronal damage, resulting in neuropathic pain. Neuronal dysfunction disrupts the balance between non-painful and painful stimuli by impairing the inhibitory or excessively stimulating the nociceptive pathway. This dysfunction induces pain in the absence of nociceptor involvement (Yagihashi et al., 2007).

Elevated glucose levels can lead to the overexpression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kß) and the mitogen-activated protein kinase (MAPK) pathway, initiating an inflammatory response. In addition, this hyperglycemic state triggers the release of inflammatory cytokines, such as transforming growth factor-beta (TGF-ß), cyclooxygenase-2 (COX-2), and tumor necrosis factor-alpha (TNF-α) in the sciatic nerves, resulting in mechanical allodynia and hyperalgesia (Ahmad and Hoda, 2020). *Alpinia calcarata*, a natural compound, demonstrates significant anti-inflammatory properties without impacting the immune system (Suryavanshi and Kulkarni, 2017). It has been shown to mitigate nociceptive pain by inhibiting the inflammatory response (Padgaonkar et al., 2017). Treatment with *Alpinia calcarata* has been observed to significantly reduce thermal hyperalgesia, mechanical hyperalgesia, and mechanical allodynia in diabetic animals. This attenuation of neuropathic pain may be attributed to inhibiting the NF-kß pathway, subsequently disrupting the inflammatory cascade.

The accumulation of reactive oxygen species (ROS) and dysregulated polyol flux elevate the activity of aldose reductase and sorbitol dehydrogenase in peripheral nerves (Yagihashi et al., 2007). This increase in ROS and sorbitol in the sciatic nerves disrupts nerve conduction velocity by inactivating Na+/K+ ATPase activity (Ahmad and Hoda, 2020). Moreover, the overexpression of NF-kß, resulting from elevated protein kinase C (PKC), ROS, and advanced glycation end products (AGEs), contributes to the degeneration of peripheral neurons through leukocytic infiltration and the reduction of neuronal growth factor (NGF), TNF-α, IL6, and IL1ß (Sugimoto et al., 1997).

Alpinia calcarata has demonstrated anti-inflammatory and analgesic effects by inhibiting the TNF-α and NF-kß pathways (Suryavanshi and Kulkarni, 2017). Treatment with *Alpinia calcarata* significantly improved motor and sensory nerve conduction velocities in diabetic rats, potentially due to its neuroprotective effect through the inhibition of oxidative stress (Padgaonkar et al., 2017). The generation of ROS in neuronal cells initiates a pro-apoptotic cascade. It releases inflammatory mediators, depleting antioxidant enzymes, such as malondialdehyde, glutathione, catalase, and superoxide dismutase. The disruption of these antioxidant enzymes results

in mitochondrial dysfunction, demyelination of neurons, and extracellular matrix accumulation (Ahmad and Hoda, 2020). *Alpinia calcarata* treatment has been observed to normalize antioxidant enzyme levels and decrease oxidative stress in the sciatic nerves. This normalization of oxidative enzymes helps prevent demyelination and confers neuroprotection.

Alpinia calcarata treatment demonstrated a significant protective effect on the sciatic nerves. Histological examination using H&E staining showed preserved nerve morphology and intact myelin sheaths. It reduced inflammatory cell infiltration in the *Alpinia calcarata*-treated group compared to the disease group, which displayed degenerative changes like axonal swelling and myelin loss. Masson's trichrome staining exhibited a more organized collagen distribution in the treated group, suggesting a possible preventive effect against fibrotic changes. Furthermore, Kulchitsky-Pal staining revealed decreased neurofibrillary tangles in the *Alpinia calcarata*-treated group, implying a potential neuroprotective role. Overall, these findings indicate that *Alpinia calcarata* may be beneficial in preventing nerve damage and neurodegeneration.

Conclusion

The present study provides compelling evidence that the ethanolic extract of *Alpinia calcarata* Roscoe rhizome exerts significant protective effects against streptozotocin-induced diabetic peripheral neuropathy in rats. Our findings demonstrate that the extract ameliorates hyperglycemia, alleviates oxidative stress, and promotes nerve regeneration, thereby mitigating the structural and functional damage caused by diabetic peripheral neuropathy. These results suggest that bioactive compounds in *Alpinia calcarata* Roscoe rhizome may serve as potential therapeutic agents for treating and managing diabetic peripheral neuropathy. However, further research is warranted to elucidate the precise molecular mechanisms underlying the protective effects of the extract.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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