JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/46263
RESEARCH ARTICLE

Investigation of Neuroprotective Qualities of *Saraca asoca* Methanolic Leaf Extract Against Diabetes-induced Peripheral Neuropathy in Rat Model

M. G. Hariprasad and M. G. Mubasheera*

Department of Pharmacology, KLE College of Pharmacy, Bangaluru - 560010, Karnataka, India; mubasheeramgphd@gmail.com

Abstract

Background: One typical consequence of diabetes is diabetic peripheral neuropathy, which damages nerves and impairs pain perception, especially in the legs and feet. **Aim**: The objective of this research was to look into how *Saraca asoca* methanolic leaf extract protected rats from diabetic neuropathy caused by streptozotocin. **Methods:** (6 per group): Two treatment groups received oral administration of *S. asoca* methanolic leaf extract (200 and 400 mg/kg) for four weeks, a normal control group, and a diabetic control group. Elevated blood glucose levels are the cause and confirmatory factor for diabetes. One dosage of streptozotocin (55 mg/kg) administered intraperitoneally caused diabetes, which can lead to diabetic persistence and peripheral neuropathy. **Results:** Diabetes was induced and confirmed by elevated glucose levels, hence the findings showed that the rats treated with *S. asoca* had significantly lower blood glucose levels and higher body weights than the diabetic group. The research found that *S. asoca* treatment significantly improved behavioural parameter in rats. Additionally, the extract demonstrated protective benefits against oxidative stress, nociception and restore glycosylated haemoglobin. By using modern ultrasound technology like histopatholgy using Aperio Image Scope, enables real-time dissection of the sciatic nerve which revealing reduced nerve damage, degeneration of the myelin sheath and lower incidence of neurofibrillary tangles in treated animals. **Conclusion:** This study shows that, potentially as a result of its antioxidant qualities and capacity to reduce oxidative stress, *S. asoca* treatment has protective benefits against diabetic peripheral neuropathy in rats. According to the results, *S. asoca* methanolic leaf extract could be an effective therapy for diabetic peripheral neuropathy. These findings open up new avenues for investigation into the mechanisms behind these protective benefits.

Major Findings: *Saraca asoca* leaf extract at 400 mg/kg significantly mitigated diabetic peripheral neuropathy in rats by enhancing antioxidant defenses, reducing blood glucose levels, and preserving nerve structure. These findings suggest its potential as a promising natural therapeutic agent for managing diabetic neuropathy.

Keywords: Diabetes, Eosin, Hematoxylin, Oxidative Stress, Peripheral Neuropathy, *S. asoca,* Streptozotocin

1. Introduction

Blood sugar levels are raised by diabetes mellitus, a severe global health problem that is defined by abnormalities in insulin synthesis, action, or both[1]. Diabetes is becoming more and more common worldwide, impacting millions of individuals[2]. As stated WHO figures from 2019, worldwide diabetic cases are expected to reach 783 million by 2045, with India having the second-largest number at over 124 million diagnosed by that year, compared to 1.5 million fatalities in 2019[3]. Uncontrolled diabetes can lead to complications like both macrovascular (heart attack, stroke, and peripheral disease) and as well as microvascular (neuropathy, retinopathy and nephropathy) conditions significantly raise morbidity and death rates[4]. Peripheral neuropathy, a condition affecting hand and foot discomfort, numbness, and

*Author for correspondence

Article Received on: 07.10.2024 Revised on: 10.03.2025 Accepted on: 12.03.2025

weakness are symptoms of a disorder that affects peripheral nerves outside the brain and spinal cord, which also affects digestion and urine[5].

The disorder known as Diabetic Peripheral Neuropathy (DPN) is characterized by metabolic abnormalities in the peripheral nerve system that impact both the somatic and autonomic branches. These abnormalities are brought on by elevated polyol pathway activity and changed protein kinase C activity[6].

Symptoms of DPN are known to include muscle weakness, cramps, muscle twitching, muscular atrophy, foot ulcers, osteoporosis, periungual erythema and nail telangiectasis, acanthosis nigricans, eczema, pruritus, xanthelasma, and xeroderma pigmentosum diabetic dermopathy, changes such as hair loss, fragility, or sparsity, or alopecia areata cause variable patterns of hair damage[7-9]. According to the American Academy of Neurology, Pregabalin the first-line anticonvulsant drug in patients with DPN, along with other medications like Venlafaxine, Duloxetine, Amitriptyline, Gabapentin, Valproate, Opioids, and Topical capsaicin. A 30% reduction in pain intensity is considered a meaningful reduction. Currently, available antidepressant drugs such as Opioids (morphine sulphate, oxycontin), anticonvulsants (carbamazepine, gabapentin, and valproate sodium), amitriptyline, desipramine, duloxetine, and venlafaxine. Opioid-like analgesics (dextromethorphan, tapentadol, and tramadol), because of these medications side effects, which greatly increase patient noncompliance and therapy failure, natural substances are being used as replacement therapeutic agents for neuroprotective care[10].

Herbal treatments were utilised methodically in the ancient Ayurvedic medical system. Ashoka, also known as *Saracaasoca* (Roxb) Wilde, is an indigenous plant with several traditional meanings that belongs to the Legume subfamily *Caesalpinaceae*. This plant's whole is thought to be pharmacologically significant, and this has been specifically utilized to address several gynecological issues, such as dysfunctional uterine hemorrhage, menorrhagia, and leucorrhoea[11-13].

A prior study found that the *S. asoca* leaf extract includes a variety of phytoconstituents, including stearic, palmitic, linoleic, and oleic acids, and has excellent antioxidant activity[14]. Gallic acid, leucocyanidin, lucocyanidin, palmitic, stearic, linolenic, linoleic, cyanidin-3, 5-diglucoside, pelargonidin-3, and quercetin are among the compounds that have been identified[15]. The ethanol extract of *S. asoca* leaves used in the phytochemical screening test contained alkaloids, saponins, phenols, tannins, and flavonoids[16]. Alkaloids, flavonoids, terpenoids, and steroids are among the secondary metabolite chemicals found in *S. asoca* leaf methanol extract[14]. Test samples consist of fractions and crude extracts, as well as pure compounds derived from natural components[17]. *S. Asoca* extracts biological activities, including potent and anti-inflammatory qualities, which might help explain its possible protective benefits against diabetes and its consequences. Additionally, *S. asoca* has been demonstrated in earlier research to exhibit antihyperlipidemic and hypoglycemic actions in diabetic rats caused by STZ, indicating a possible function in the management of diabetes[14].

Referring to the areas outside of the central nervous system the brain and spinal cord to the entire body through a network of forty-three motor and sensory nerves known as the peripheral nervous system. It regulates sensation, movement, and motor coordination[18].

Peripheral nerves consist of fascicles of axons ensheathed by Schwann cells, grouped into fascicles of variable numbers. They have multiple layers of connective tissue, blood vessels, and nerves, and are surrounded by Schwann cells[19]. Peripheral neuropathy, triggered by pain signals and vasa nervorum constriction, affects blood flow and inflammation, but not a diagnosis for conditions like vitamin B12 deficiency neuropathy, diabetic neuropathy, alcohol-induced neuropathy, and chemotherapy-induced neuropathy[20].

This study provides an overview and illustrations of the peripheral nerves primary structural and ultrastructural characteristics. Furthermore, peripheral nerves, which distribute their branches and ends throughout the whole body, might be impacted by a variety of disorders. According to the literature review, no studies on DPN have been conducted to far utilizing SAMLE. Thus, this study reveals antioxidant activity as a potential health benefit and validates the neuroprotective potential of phytoconstituents in *S. asoca* utilizing histopathological techniques. Thus, the purpose of the current study was to learn more about

the possible impact of SAMLE on STZ-induced DPN in rat models.

2. Materials and Methods

2.1 Chemicals and Reagents

Streptozotocin (Sigma-Aldrich® Lab, Karnataka, India), Insulin, Sodium Citrate, Citric Acid, Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium Chloride, Glutaraldehyde, Phosphate Buffer, Phenazine Methosulphate, Nicotinamide Adenine Dinucleotide (NADH), Glacial Acetic Acid, N-Butanol, Tris Buffer, Potassium Dichromate Acetate, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Disodium Hydrogen Phosphate, 5,5′-Dithiobis-(2-Nitrobenzoic Acid) (DTNB), Methanol, Normal Saline, Malondialdehyde, glutathione, and Catalase. The measurements were conducted using a commercially available kit and obtained from VASA scientific Pvt. Ltd, Bangalore, India. All of the chemical reagents utilized in this study were analytical grade.

2.2 Preparation of Plant Material

Saraca asoca leaves were gathered from the Indian Institute of Horticultural Research, Bangalore (IIHR). The plant has been verified by Dr. P. E. Rajasekharan, Principal Scientist and Nodal Officer GAC, Division of Plant Genetics Resources, verified and confirmed by the same.

2.3 Extraction of *S. asoca* Leaves

After being thoroughly cleansed under running water three times and allowed to dry in the shade, the *S. asoca* leaves were cut into small pieces using a wood grinder. Samples that had been dried were ground into a rough powder. A 3000 ml Soxhlet apparatus filled with about 250 gram of coarsely ground material was used for the extraction process, which followed a previously published procedure. Methanol was used as a solvent for three days in a continuously running method, with the boiling point being maintained at 64.7° C. Following the extraction, the solvent was collected and put to further use by evaporating it in a rota evaporator. For the animal investigation, this crude extract was diluted with water to yield final doses of 200 and 400 mg/kg body weight.

2.4 Animals

Male Wister albino rats were used in the study for weighing 200±20 gram acquired from a licensed breeder (Biogen Laboratory Pvt. Ltd), Bangalore. For the course of the study, rats were kept in a controlled environment within the research facility, with a 12-hour light/dark cycle, a temperature of 22 ± 2° C, and a humidity of 45 ± 5%. The animals were fed a healthy diet and allowed unlimited access to water. The rats were allowed to acclimate to their new environment for one week prior to the experiment starting.

2.5 Induction and Assessment of Diabetic Neuropathy

An intraperitoneal injection of 55 mg/kg (streptozotocin) STZ formulated in citrate buffer (pH 4.4, 0.1 M) was given once in order to induce diabetes. 48 hours after the STZ injection, diabetes was verified. Heparinized capillary glass tubes were used for the retro-orbital plexus procedure to collect blood samples, and the diagnostic kit method was used to assess the plasma glucose levels. For the purposes of this investigation, plasma glucose levels of rats more than 250 mg/dL were chosen. Both before to and during the trial, the subjects plasma glucose levels and body weight was assessed[22].

2.6 Evaluation of Behavioral Parameters

2.6.1 Evaluation of Body Weight and Blood Glucose Levels

In a rat model of peripheral neuropathy brought on by STZ, the study assessed body weights, fasting blood glucose levels, and plasma glucose levels both before and after treatment[22].

Table 1. Experimental design

[A] Non-diabetic Animals	
Group 1	Normal control: There is no medical intervention.
[B] Diabetic Animals	
Group 2	Diabetes ((STZ; 55mg/kg b.w, IP) control: Animals received oral gavage of purified water.
Group 3	Diabetes (STZ) + *S. asoca* low dose (200 mg/kg/day) in purified water for 4 weeks.
Group 4	Diabetes (STZ) + *S. asoca* high dose (400 mg/kg/day) in purified water for 4 weeks.

2.6.2 Thermal Hyperalgesia

The tail of the rat was immersed in hot water at 46° C and pain indicators was recorded. A 12-second latency period was set, indicating increased pain sensitivity. The tail was dried after each reaction[23].

2.6.3 Cold Hyperalgesia

Cold water of 4° C was dipped into a rat tail, and a 10-second observation showed a swift tail retraction, indicating cold hyperalgesia[24].

2.6.4 Rota-rod Performance

The Rota-rod evaluation measures rats motor coordination by allowing them to stay on a revolving 75 mm diameter rod for five minutes. The rod rotates at a rate of 25 revolutions per minute, and the rat was placed on it for the assessment. Three practice sessions were conducted, recording each rats successful duration on the rod[25].

2.6.5 Grip Strength Performance

The study evaluated the grip strength of rat models using a grip strength assessment. The animals underwent an acclimatization procedure, were hung by their tail, and then forced to relinquish their grip on a grid, with force output measured in kilos or newton[26,27].

2.6.6 Narrow Beam Walk Performance

The study evaluated the grip strength of rat models using a narrow beam walk assessment, measuring foot slip errors and the time required to cross a fixed wooden plank[28,29].

2.6.7 Hanging Wire Performance

The hanging-wire test measures neuromuscular strength in animals by placing rat on a wire lid, recording the longest latency, and allowing previously trained animals to hang upside-down[30].

2.7 Oxidative Stress Parameter

Following the experiment, sciatic nerves were separated and homogenized in ice-cold phosphate buffer (0.1M, pH 7.4) using a probe homogenizer. The quantity of protein in the tissue homogenate was measured using a folin phenol reagent[31]. Malondialdehyde (MDA) was used to measure lipid peroxidation[32]. Reduced glutathione (GSH) levels were measured in order to identify tissue sulfhydryl groups[33]. Catalase activity was determined by measuring the rate of hydrogen peroxide degradation per minute[34]. Additionally, the glutathione, catalase, and malondialdehyde activities were determined using the following formula:

1. Concentration of MDA = absorbance (test)-absorbance (blank)/1.56x10.00.000
2. Concentration of GSH = (absorbance of test-absorbance of blank) ×2×3.1× df/ (6.22) (0.05)
3. Concentration of CAT = 2.3At× initialx1.63x10-3 / final

2.8 Histological Examination of Sciatic Nerve

Histological examinations of sciatic nerves were carried out by recognized standard operating procedures. After a meticulous dissection of a sciatic nerve specimen, a section of the nerve was embedded in paraffin and preserved in a 10% glutaraldehyde solution. Using a rotary microtome, serial tissue slices with a thickness of 5 m were produced. The tissues were then stained using eosin and Mayer's hematoxylin for overall morphology and visualized by conventional pathological laboratory methods[35].

2.8.1 Method of Histopathology

The study involved a detailed examination of sciatic nerves through standard histological procedures, including dissection, preservation, and staining. Serial tissue sections were prepared and stained with eosin and Mayer's hematoxylin to observe overall morphology. A comprehensive literature review was conducted to understand the microanatomical architecture and microcirculation of peripheral nerves, providing a foundation for consistent image scope assessment. To establish specific parameters for sonographic evaluation, the study carefully described histological features of peripheral nerves in both acute and chronic stages of neuropathy. Additionally, high-frequency ultrasound probes and advanced ultrasound equipment were used to perform Aperio image scope examinations, enabling precise diagnostic analysis of peripheral nerve tissue.

2.8.2 Histopathological Preparation and Evaluation

The preparation of histopathology was outsourced. In summary, tissue samples obtained 0.5 cm from

the sciatic nerve injury were maintained in a 0.4 M PBS solution (pH 7.4) with 2.5% glutaraldehyde (Merck KGaA) for 4 hours at 4˚ C. After being fixed for one hour at 4˚C in 1% OsO_4, tissue samples were then passed through a series of escalating alcohol concentrations before being embedded in epoxy resin. Tissue sections were cut into slices that were 0.5 µm thick using a Leica Reichert ultra cut in ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Hematoxylin and eosin stain was then applied to the sections for 30 seconds at 50-75˚C according to the manufacturers protocol, prior to morphologyical and morphometric analyses[36]. The study measured the number of myelinated nerve fibers, nerve fiber diameter, and myelin sheath thickness in five distinct areas of the sciatic nerve sections using morphometric analysis[37]. The slides were analyzed using the analysis research software Aperio image scope x64, using light microscope (Leica Biosystems, Nussloch, Germany) at various magnifications[38].

2.9 Statistical Analyses

The Mean ± SEM was used to analyze and present the raw data, with six participants in each group. The results were compared using one-way ANOVA, and any significant differences ($p<0.05$) between the groups were subsequently examined using Dunnett's multiple comparison tests. Version 8.0.2 of Graph Pad Prism was used to analyze this data.

3. Results

3.1 Effects of SAMLE Administration on Body Weight

There was no discernible change in body weight between the diabetic control rats and the normal control rats the day before the intraperitoneal dose of STZ that caused the diabetic neuropathy in the control rats. After four weeks of intraperitoneal injection of STZ, the body weight of diabetic control rats was significantly lower than that of normal control rats as presented in Table 1. Continuous therapy with SAMLE (200 mg/kg and 400 mg/kg) for 28 days significantly and dose-dependently improved the lowered body weight when compared to diabetic control rats, which is presented in Figure 1.

3.2 Effects of SAMLE Treatment on Behavioral Parameters

In both hot and cold water tail immersion tests, the diabetic control group significantly outperformed the normal group in terms of response latency and tail withdrawal delay ($p<0.001$). In contrast to the group of people with diabetes, a four-week SAMLE therapy at all examined dosage levels significantly offset the decrease in latency. The high dose of *S. asoca* has increased comparatively in the rota-rod, hanging wire, grip strength, and narrow beam walk behavioural tests, which support the understanding of the neuromuscular strength of animals before and after the treatment, which shows an increase in the strength of animals are depicted in Figure 2.

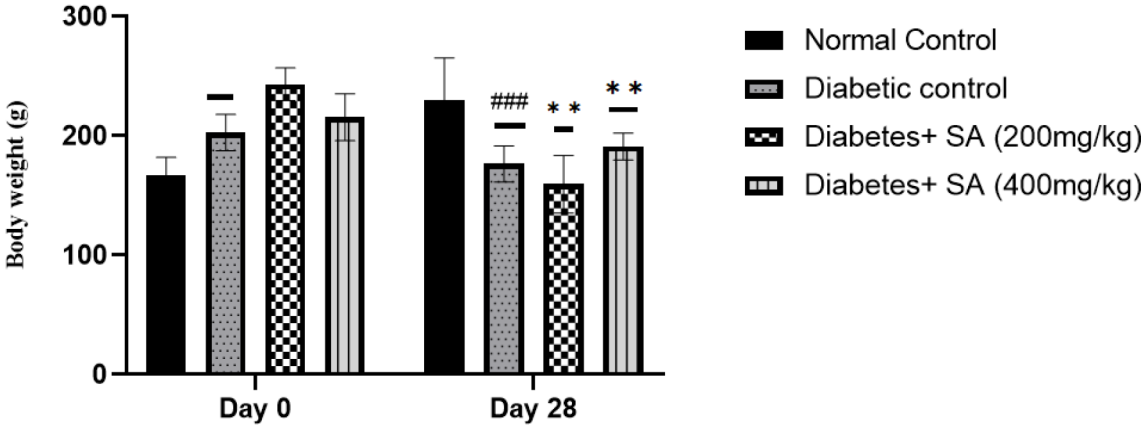


Figure 1. SAMLE treatment on STZ-induced peripheral neuropathy in rats: Body weight on the injection day (Streptozotocin) and after 0 days and 28 days.
Data: Mean ± SEM (n=6). ### $p<0.001$ *vs.* normal; *$p<0.05$, **<p0.01, ***$p<0.001$ *vs.* diabetic control.

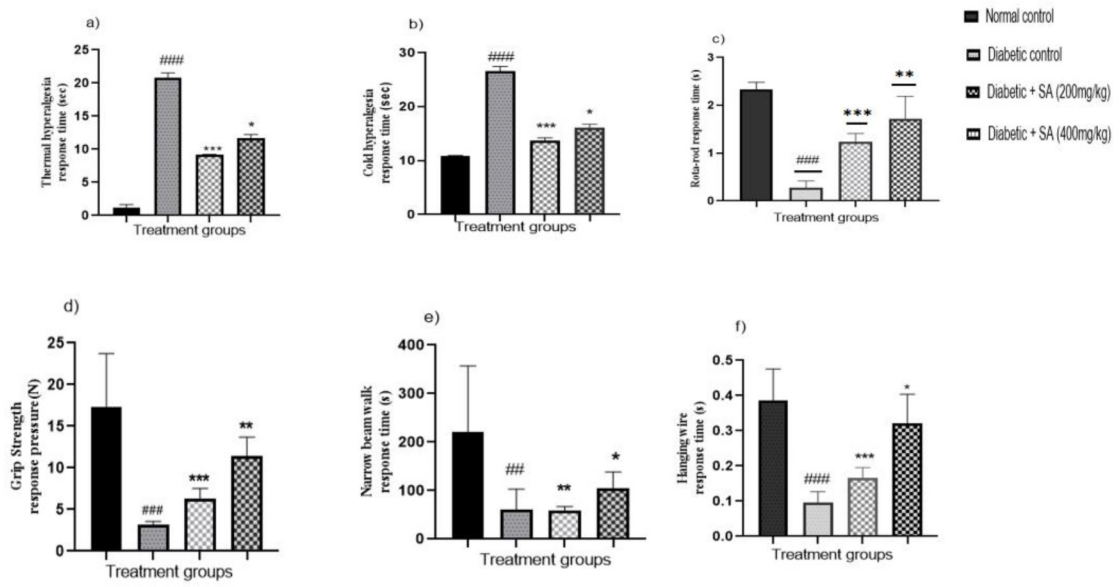


Figure 2. SAMLE treatment on STZ-induced peripheral neuropathy in rats: **(A).** Thermal hyperalgesia; **(B).** Cold hyperalgesia; **(C).** Rotarod test; **(D).** Grip strength test; **(E).** Narrow beam walk test; **(F).** Hanging wire test.
Data: Mean ± SEM (n=6). ###p<0.001*vs.* normal; *p<0.05, **<p0.01, ***p<0.001 *vs.* diabetic control.

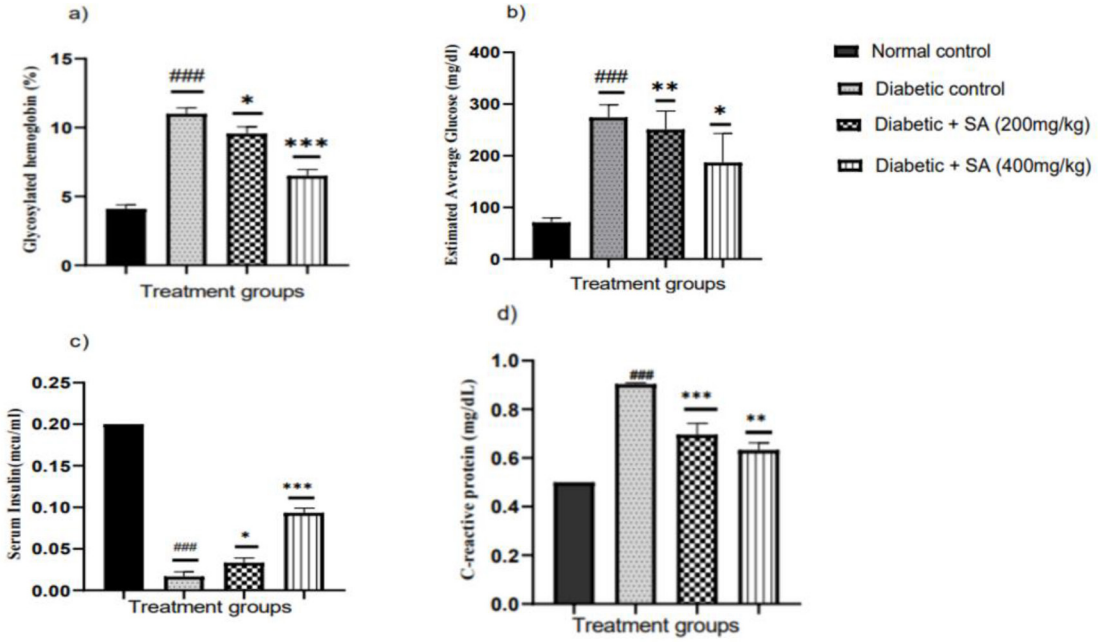


Figure 3. SAMLE treatment on STZ-induced peripheral neuropathy in rats: **(A).** Glycosylated hemoglobin; **(B).** Estimated average glucose; **(C).** Serum insulin; **(D).** C-reactive protein.
Data: Mean± SEM (n=6). ###p<0.001 *vs.* normal; *p<0.05, **<p0.01, ***p<0.001 *vs.* diabetic control.

3.3 Effects of SAMLE on Hematological Parameters in STZ-induced Rat Model

The hematological results, including glycosylated hemoglobin (%), estimated average glucose (mg/dL), serum insulin (MCU/ml), and C-reactive protein (mg/dL) are depicted in Figure 3. Except for the disease control group, all groups receive *S. asoca* therapy for 28 days. However, the low-dose (200 mg/kg) (p<0.001) groups measurements have significantly improved compared to the control group with diabetes except in serum insulin level.

3.4 Effects of SAMLE Therapy on Oxidative Stress Markers

In contrast to animals without diabetes, the diabetic control group showed a large increase in oxidative stress in sciatic nerve homogenate, as demonstrated by a significant rise in malondialdehyde levels and a drop in glutathione and catalase concentrations. *S. asoca* therapy at 200mg/kg and 400mg/kg significantly reduced malondialdehyde levels ($p<0.001$, respectively) compared to the diabetic control group. Glutathione levels increased significantly after *S. asoca* treatment at dosages of 200mg/kg ($p<0.05$) and 400mg/kg ($p<0.01$). Furthermore, catalase concentration increased significantly following *S. asoca* therapy in contrast to the control group with diabetes as depicted in Figure 4.

3.5 Histopathology of Sciatic Nerve Injury

The rats were put to sleep with diethyl ether at the end of the immobilization period, and the sciatic nerve was carefully removed and depicted in Figure 5. The sciatic nerve tissues were analyzed using hematoxylin and eosin staining on semi-thin sections from all groups. Microscopic examination of sciatic nerve sections revealed under normal circumstances maintaining normal morphological structure shown in Figures 6 and 7. Additional to illustrate the harm brought on by STZ induction, pictures of sciatic nerve injury were

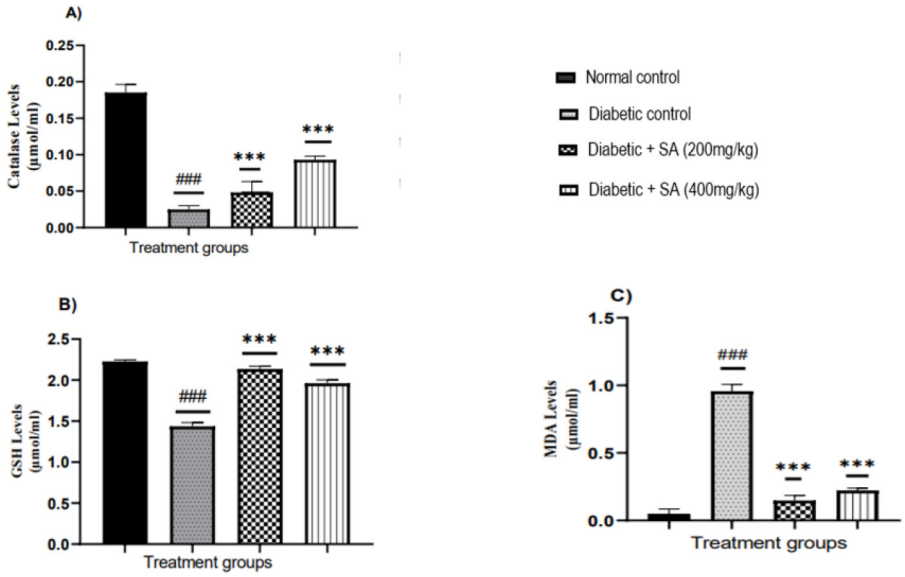


Figure 4. SAMLE leaves treatment on STZ-induced peripheral neuropathy in rats: **(A)**. Catalase; **(B).** GSH; **(C).** MDA antioxidant.

Data: Mean ± SEM (n=6). ###$p<0.001$ *vs.* normal; ***$p<0.001$ *vs.* diabetic d control.

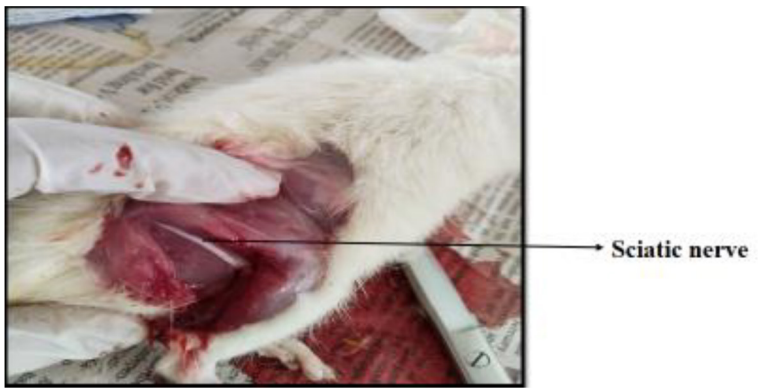


Figure 5. Sciatic nerve identification and isolation from rat model.

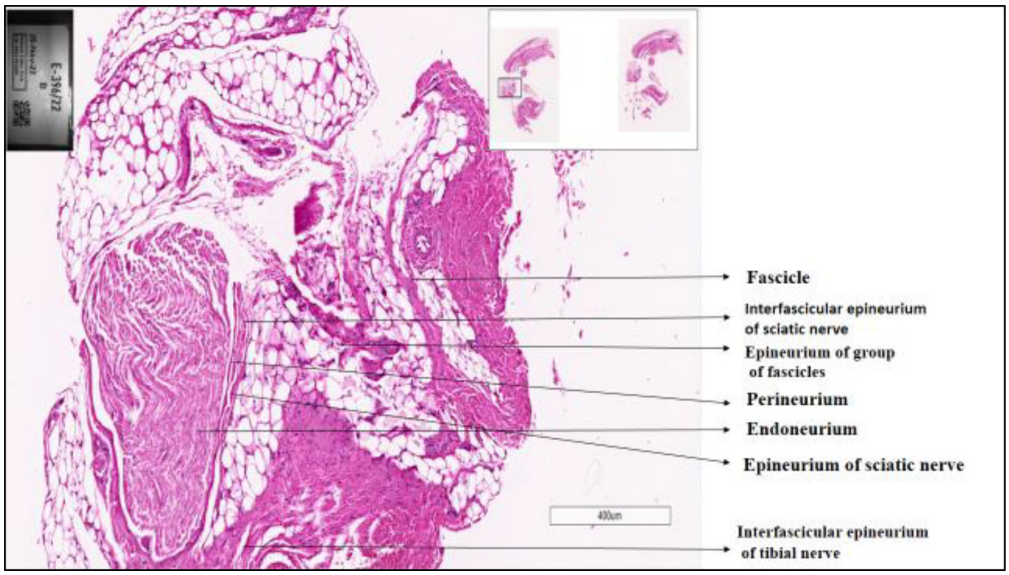


Figure 6. Histopathological patterns of sciatic nerve at the level of popliteal fossa.

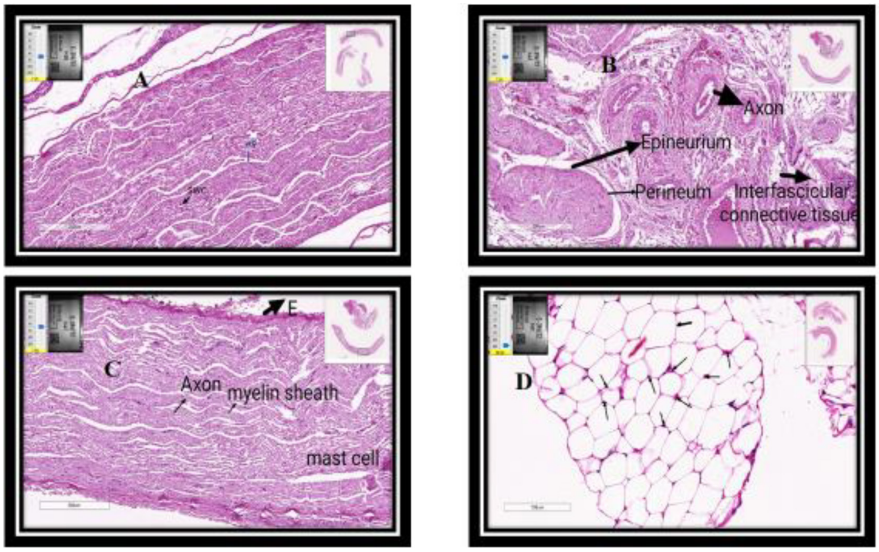


Figure 7. Sections of sciatic nerve tissue were stained with hematoxylin and eosin, and the findings are shown in typical pictures from the normal control group. **(A).** Normal histology in view of the longitudinal sections of the sciatic nerve in normal control group, the Nerve Fibers (NF) are bundled, parallel, wavy, uninterrupted and in bundle with SWC (Schwann cell nuclei. **(B).** Illustration of the perineurium and epidermis surrounding a nerve fiber bundle in a transverse section through the sciatic nerve. The transverse section of the inset displays individual nerve fibers along with their surrounding myelin and core axon. **(C).** The sciatic nerves longitudinal segment demonstrates the myelin sheath around the axon. Rat peripheral nerves frequently contain a few sporadic mast cells. **(D).** Showing the endothelial polygonal cells with Schwann cell.

displayed. Most myelinated axons are degenerated, with expansion, invagination, swelling and disruption of the myelin sheaths typical concentric lamellar structure depicted in Figure 8.

Under normal circumstances, a peripheral nerve encompasses bundles of nerve fibers (axons) and has various connective tissue coverings (superficial epineurium, perineurium, and endometrium) (Hematoxylin and Eosin stain, 400µm)

The most important histologic findings of the peripheral nerve during the acute phase is progressive edema depicted in Figure 9. Endoneurial capsules

become hyper-permeable to fluids, proteins, and mucosal substances—more especially, endoneurial glycosaminoglycans-when the blood-nerve barrier breaks down. Axonal lining injury and myelinolytic activity of mast cell-released amines and proteases dramatically increase endothelial dysfunction in endoneurial arteries intra-fascicular edema causes axons to partially break and demyelinate, allowing for the identification of spatially displaced axon bundles in the perineurial region that are surrounded by injured Schwann cells. The perineural part of the blood-nerve barrier may become histologically involved in mechanical and ischemia injury. Partial stoppage of the perineurium can also be linked to endoneurial edema. Damage to peripheral nerves causes metabolic alterations that may result in apoptosis, neuronal edema, and intense neutrophil infiltration.

Prolonged exposure to alcohol-containing fixatives or drying solutions, such as putting PNS tissues on

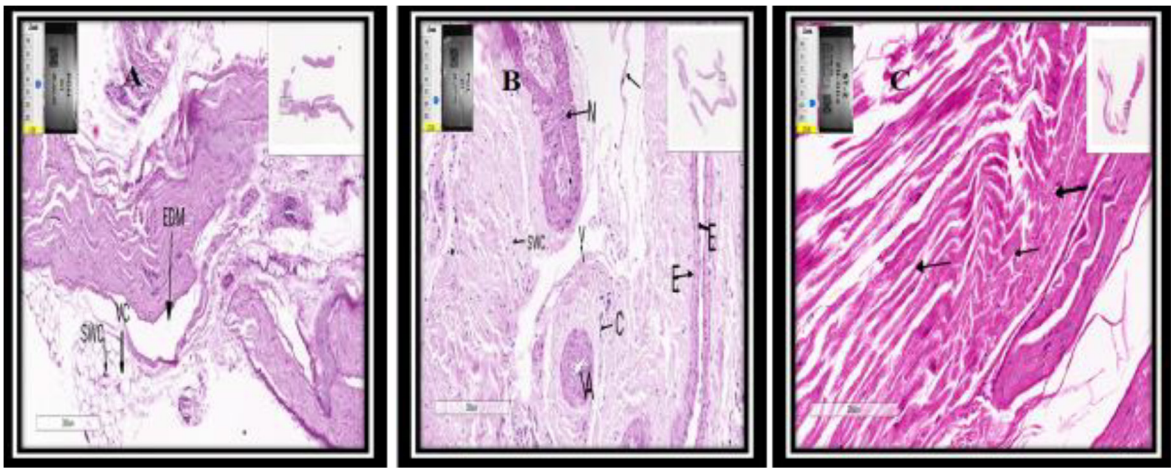


Figure 8. Sections of sciatic nerve tissue were stained with hematoxylin and eosin, and the findings of the diabetic control groups, nerve damage analysis are displayed in example pictures. **(A).** Interruption in nerve fiber continuity, edema (EDM), diffuse vacuole (V), pronounced hyperthorphia in SWC 300; **(B).** Sciatic nerve displaying the dispersed Schwann cell nuclei (arrows) and copious amounts of eosinophilic myelin that are typical of the most frequently used PNS sample in animal research. The distribution of spinal nerves (N) and their branches is linked to veins and arteries (A). E stands for epineurium, proliferation and regeneration of SWC H and E x 200; **(C).** An excessively handled or poorly fixed nerve during necropsy resulted in a procedural artefact. Many nerve fibers have a fuzzy cytoarchitecture (arrow).

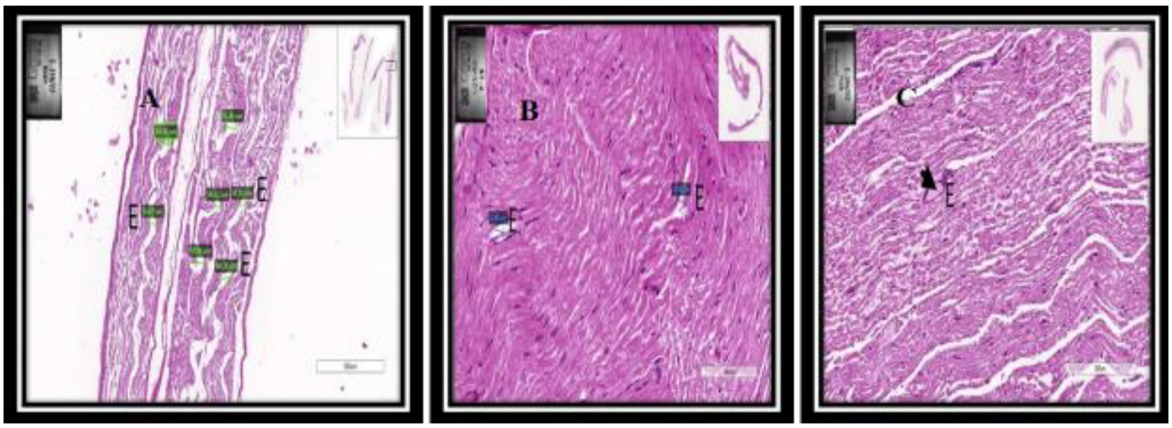


Figure 9. Histopathological configuration of intra-fascicular edema. **(A).** The diabetic control group displayed considerable axonal enlargement, nerve fiber disturbance, and an increase in Schwann cells 500, **(B).** and **(C).** Both the low dosage (200 mg/kg) and high dose (400 mg/kg) of *S. asoca* treatment groups showed improvements in nerve fiber organization and it is possible to concurrently observe parallel, extremely rarefied bundles of Schwann cells and axons at several locations inside the same fascicle.

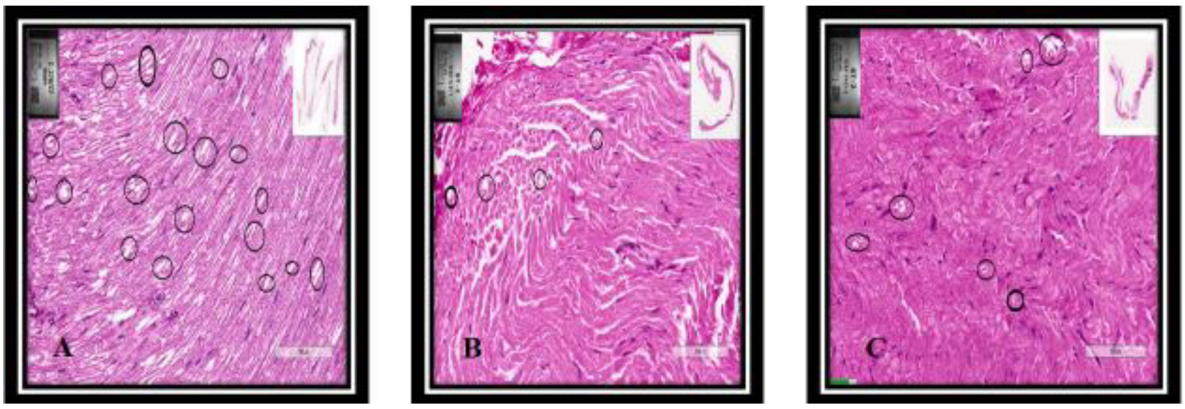


Figure 10. Myelin sheath vacuolation as a processing artefact. **(A).** Ellipse marking are showing the presence of vasculization in diabetic control group rats. **(B).** and **(C).** Ellipse markings are comparatively less then control group in low dose treatment with *S. asoca* leaf extract on H and E X100µm. The vacuolation extent increases with alcohol concentration and duration in an alcohol-containing fixative or automated tissue processor, as shown in the arrows.

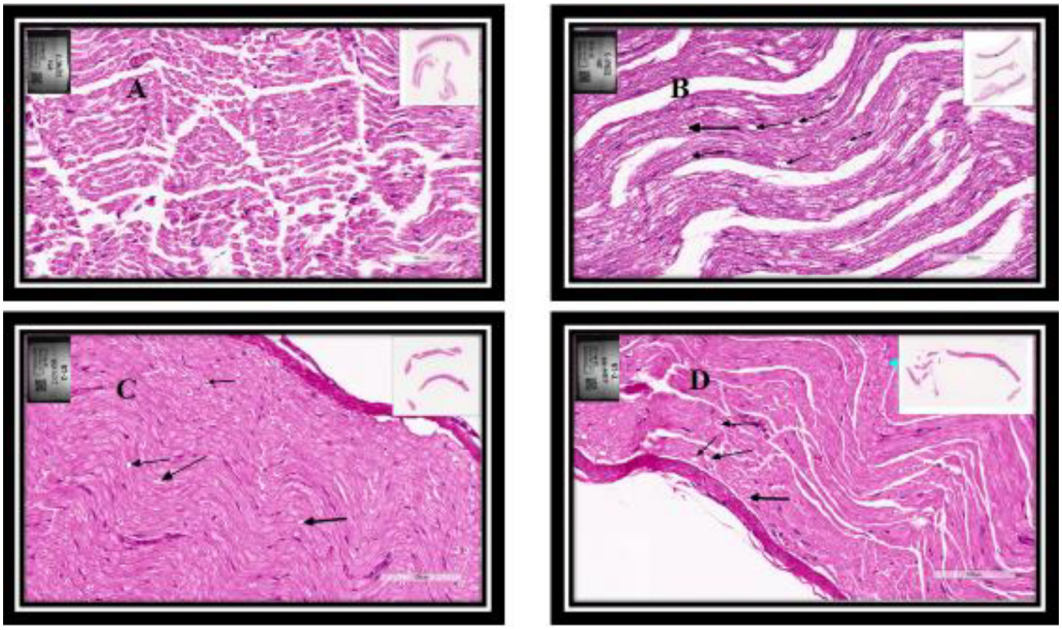


Figure 11. Effect of SAMLE (200 and 400mg/kg, oral) on sciatic nerve- hematoxylin and eosin staining **(A).** Normal control depicts the perineurium and epidermis surrounding a nerve fiber bundle in a transverse section through the sciatic nerve, revealing individual nerve fibers and their surrounding myelin and core axons with normal nerve morphology **(B)**. Diabetes control: Reveals reduced nerve fiber density in the endoneurium, increased vascular proliferation, severe axon and myelin degeneration, and decreased Schwann cells in diabetic rats **(C).** *S. asoca* low dose treatment 200mg/kg shows mild axon degeneration **(D).** *S. asoca* high dose treatment 400mg/kg shows moderate degeneration of nerve fibers in the sciatic nerve. Damaged Granular debris is created from nerve fibers, from eosinophilic to amphophilic, confined within intact basement membranes. The scale bar represents X 200 µm, 300 µm, and 400 µm.

a tissue processor for the whole weekend, may cause myelin vacuolation or the formation of insoluble neurokeratin in the nerves, as seen in Figure 10. It has been demonstrated that hydropic degeneration, or the buildup of intracellular fluid, is the source of treatment-related vacuolation of dorsal root ganglion neurons. Since neuronal vacuolation is frequently observed in control animals of different species, it is important

to exercise extreme caution and prudence when interpreting it as an artifact or treatment-related effect. Hence the overall effect of SAMLE (200 and 400mg/kg, oral) on sciatic nerve, is depicted in Figure 11.

4. Discussion

Apoptosis is generally acknowledged to have a role in tissue change and cell loss in peripheral neuropathies, a disease marked by nerve injury and tissue degeneration[39]. Nearly 50% of diabetic patients experience neuropathic pain, a typical consequence of the disease that manifests as pain, hyperalgesia, and spontaneous allodynia. Recent studies suggest that diabetes increases risk of peripheral neuropathy, a condition that damages the sciatic nerve, causing numbness, tingling, and loss of sensation, which can be exacerbated by other health conditions[40]. The current study investigates *S. asoca*'s possible beneficial impact on diabetes-induced neuroprotective activity. The demand for herbal remedies has surged due to their fewer side effects, efficacy, and lower treatment costs. The WHO approves plant-based natural drugs for various diseases, including diabetes[41]. *S. asoca*, a plant with numerous phytoconstituents, has numerous health benefits and improving *S. asoca* intake could potentially enhance peripheral neuroprotective activity. The research examined the effects of two doses of SAMLE orally, a lower dose of 200mg per kg body weight and a higher dose of 400mg per kg body weight, on peripheral neuropathy, a condition caused by increased diabetes risk, affecting the sciatic nerve. Diabetes rats experience weight loss due to muscle wasting and tissue protein and fat loss[42]. SAMLE low-dose and high-dose-treated diabetic animals show marginal weight increases compared to diabetic controls, possibly due to protective effects on muscle wasting, improved glucose uptake, insulin secretion, and glycemic control, and reversal of gluconeogenesis. Uncontrolled diabetes is linked to weight loss, increased C-reactive protein, glycosylated hemoglobin, and estimated average glucose levels, making individuals at high risk for microvascular and macrovascular complications[43]. Within the current investigation, there was a significant rise in the diabetic control group in CRP, estimated average glucose and glycosylated hemoglobin level, while a significant decrease in serum insulin level, was found. Hyperlipidemia, a common complication of diabetes, is characterized by increased CRP, glucose, and glycosylated hemoglobin levels[44]. Treatment with methanolic extract of SAMLE significantly normalizes these lipid abnormalities and improves overall health. The study evaluated behavioural parameters like hyperalgesia, cold hyperalgesia, grip strength, and narrow beam walk in an *in-vivo* setting. The lumbosacral plexus's anterior and posterior divisions provide fibers to the nerve, which derives from spinal nerves L4 through S3[45]. Diabetes-induced diabetes in rat models was used to study neuropathic pain with hot and cold hyperalgesia[46].

Diabetic neuropathic pain is caused by reduced motor nerve conduction velocity, decreased NA, decreased K ATPase activity, cyclooxygenase activation, adenosinergic and opioidergic system modulation, and increased oxidative stress. This reduced NA, K ATPase activity in nerve tissue is linked to the development of diabetic neuropathy[47]. Furthermore, there is a decrease in NA and K ATPase activity in the brain, cardiomyocytes, and erythrocyte membrane of diabetic mice[48]. In diabetic rats, slower nerve conduction is associated with decreased NA, K ATPase activity in the sciatic nerve[49]. Because of physiological changes, including reduced myo-inositol and protein kinase C activity, hyperglycemia can affect the function of NA and K ATPase[50].

According to research, apoptosis is a process that causes tissue deterioration and nerve injury. By blocking this process, diabetes neuropathy can be avoided or even encouraged. Apoptosis has an impact on peripheral neuropathies, which include injury to the nerves and degeneration of the nerve tissue[51]. Like other neuropathies, DPN is most likely the result of degeneration. Withdrawal response in hyperalgesia tests to heat and cold stimuli is caused by the spinal motor reflex, which is activated by substance P being released in the spinal cord[52]. This research investigates the preventive effects of SAMLE on diabetes-induced peripheral neuropathy in a rat model.

STZ-induced diabetes in rat models is utilized for neuropathic pain, incorporating hot and cold hyperalgesia models that mimic symptoms of diabetes[53]. In the SAMLE, the loss of Ad fibers in peripheral neuropathic pain and the decreased sensitivity of cold receptors were both reversed. Rotarod, hanging, and

muscle grip strength tests were also conducted, which are reliable methods of assessing muscle strength and motor skills. Retention time was considerably lower in STZ-induced neuropathic rats than in normal control animals, suggesting a reduction in muscular grip. In the treatment group, animals were able to remain on the rota-rod for significantly longer periods than STZ-induced rats, and their muscle strength significantly increased in comparison to the disease control group, demonstrating that SAMLE extracts were neuroprotective against diabetic neuropathy caused by STZ in rats.

In addition, antioxidant activity like catalase, glutathione, and malondialdehyde levels were estimated. Prolonged sciatic nerve constriction can cause oxidative stress, leading to increased malondialdehyde, advanced oxidation protein products, and decreased glutathione and catalase levels. This stress causes the production of superoxide and hydroxyl radicals as well as the autoxidation of monosaccharides, which is a significant cause of neuropathy in diabetes and chronic hyperglycemia. Reactive oxygen species are produced during pain transmission, and oxidative stress is associated with death in many cell types, which modifies signal transduction and genomic processes[54]. Increased peroxidation of membrane lipids, oxidative damage to macromolecules, modifications to the signal transduction pathway, changes in the cellular redox potential, and glutathione depletion are some of the ways that oxidative stress can cause apoptosis[55]. Research has indicated that elevated oxidative stress triggers caspase 3 and has a role in diabetic neuropathy. Apoptosis inhibition can either encourage regeneration or stop nerve degeneration. By inhibiting apoptosis, antiapoptotic substances can help stop hyperalgesia in diabetics[56].

Cytochrome C is released as a result of mitochondrial enlargement and malfunction brought on by hyperglycemia. Diabetic neuropathy also exhibits similar structural change[57]. The function of apoptosis in tissue modification and cell loss is thought to be responsible for peripheral neuropathies, which are typified by nerve injury and tissue degradation. From the trial like other neuropathies, DPN is a neuropathy that is most likely the consequence of degeneration, which leads to the loss of the cytoplasm and nucleus of the Schwann cell while maintaining the plasma membrane and supporting collagen to generate ghost cell[58].

The current research histopathological picture found that STZ treatment in rats reduced demyelination, Wallerian and myelin degeneration, Schwann cell decrease, and increased vascular proliferation. Fibers were degenerated and misarranged, and myelin vacuolization was observed. This implies that in diabetic sciatic nerves, apoptosis leads to axon shrinkage. Demyelination may be related to reduce protein production in Schwann cells and degradation of cytoskeleton protein, resulting to axonal loss and myelin sheath folding. Demyelination in diabetic neuropathy is due to myelin lipid peroxidation. Since SAMLE is already being used in clinical settings, antioxidants like quercetin and α-lipoic acid have been proven safe to employ as adjuvants in the treatment of diabetic neuropathy.

The research summary, suggests that SAMLE treatment can improve hyperglycemia-induced thermal hyperalgesia and reduce oxidative stress-mediated apoptosis, potentially preventing nerve damage in diabeties, thus potentially aiding in the prevention of peripheral neuropathy.

5. Conclusion

In conclusion, our data suggest that SAMLE possesses neuroprotective activity by increasing CRP, estimated average glucose, and glycosylated hemoglobin levels. In diabetic rats, it increased blood insulin levels in addition to behavioural characteristics. There was myelin vacuolization and degraded and rearranged fibers. According to recent studies, the extract lessens the structural and functional damage that DIPN produces by promoting nerve regeneration and reducing oxidative stress. *S. asoca* contains various chemical components, with quercetin dihydrate as the main active flavonoid responsible for various pharmacological effects. Thus, it might be developed as an alternative to pregabalin salts as a natural remedy for peripheral neuropathy. It may be a useful therapeutic agent for controlling and treating peripheral neuropathy, but further research is required to ensure safety and determine the best product formulation.

6. Acknowledgments

The research laboratory was provided by the faculty of pharmacy in Bangalore and KLE Academy of Higher

Education, for which the authors are grateful. The writers would especially like to thank Dr. Anand K. Soshee, Managing Director, for their valuable technical support in providing medical images and digital pathology from MIS-LBS, Bangalore. The writers would especially thank Dr Feroze Fathima for their valuable technical support in making manuscript.

7. Ethical Approvals

The Institutional Animal Ethics Committee (IAEC) of KLE College of Pharmacy in Bangalore authorized all of the protocols and techniques used in this study (permission number: IAEC/01/HP/202)

8. Author Contribution

M. G. Mubasheera: Writing-review and editing, writing-original draft, methodology, conceptualization. M. G. Hariprasad: Supervision, methodology, investigation. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

9. References

1. Grover A, Sharma K, Gautam S, Gautam S, Gulati M, Singh SK. Diabetes and its complications: Therapies available, anticipated, and aspired. Curr Diabetes Rev. 2021; 17(4):397-420. https://doi.org/10.2174/1573399816666201103144231
2. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes. 2015; 6(6):850-867. https://doi.org/10.4239/wjd.v6.i6.850
3. Diabetes. World Heart Federation. 2021.
4. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pac J Trop Biomed. 2012; 2(5):411-420. PMID: 23569941. PMCID: PMC3609313. https://doi.org/10.1016/s2221-1691(12)60067-7
5. Peripheral neuropathy [Internet]. Mayo Clinic. 2023 [cited 2024 Feb 19]. Available from:https://www.mayoclinic.org/diseases-conditions/peripheral neuropathy/symptoms-causes/syc-20352061
6. Singh R, Kishore L, Kaur N. Diabetic peripheral neuropathy: Current perspective and future directions. Pharmacol Res. 2014; 80:21-35. https://doi.org/10.1016/j.phrs.2013.12.005
7. Macedo GMC, Nunes S, Barreto T. Skin disorders in diabetes mellitus: An epidemiology and physiopathology review. Diabetol Metab Syndr. 2016; 8:63. https://doi.org/10.1186/s13098-016-0176-y
8. Nabaie L, Kavand S, Robati RM, Sarrafi-rad N, Shahgholi L, Razavi GM. Androgenic alopecia and insulin resistance: Are they related? Clin Exp Dermatol. 2009; 34(6):694-697. https://doi.org/10.1111/j.1365-2230.2008.03118.x
9. Kaur J. An overview of diabetic neuropathy. Annu Res Rev Biol. 2013.
10. Cohen K, Shinkazh N, Frank J, Israel I, Fellner C. Pharmacological treatment of diabetic peripheral neuropathy. PT. 2015; 40(6):372-388. PMCID: PMC4450668. PMID: 26045647.
11. Athiralakshmy TR, Divyamol AS, Pallath N. Phytochemical screening of *Saraca asoca* and antimicrobial activity against bacterial species. Asian J Plant Sci Res. 2016; 6(2):30-36.
12. Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K, Kudale S, Desai N. Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De. Wilde. Int J Adv Res. 2015; 3(5):318-327.
13. Verma A, Jana GK, Chakraborty R, Sen S, Sachan S, Mishra A. Analgesic activity of various leaf extracts of *Saraca indica* Linn. Pharm Lett. 2010; 2(3):35.
14. Jain A, Chaudhary J, Saini V, Sharma S. Hypolipidemic, hypoglycemic and antioxidant potential of *Saraca asoca* ethanolic leaves extract in streptozotocin induced-experimental diabetes. Int J Pharm Pharm Sci. 2013; 5(1):302-305.
15. Panchawat S, Sisodia SS. *In vitro* antioxidant activity of *Saraca asoca* roxb. De wilde stem bark extracts from various extraction processes. Asian J Pharm Clin Res. 2010; 3(3):231-233.
16. Mukhopadhyay MK, Nath D. Phytochemical screening and toxicity study of *Saraca asoca* bark methanolic extract. Int J Phytomed. 2011; 3(4):498-505.
17. Varaprasad N, Arumugam S, Suresh V, Kumar SN, Rajendar A, Madeshwaran M. Antipyretic activity of methanolic extract of *Saraca asoca* (roxb.) de wild leaves. Int J Pharm Res Dev. 2011; 3:202-207.
18. Benzo CA. Nervous system. Avian Physiology. Springer. 1986: pp. 1-36.
19. Smith BE. Anatomy and histology of peripheral nerve. Handb Clin Neurophysiol. 2006; 7:3-22. Elsevier. https://doi.org/10.1016/s1567-4231(09)70062-0
20. Teixeira MJ, Almeida DB, Yeng LT. Concept of acute neuropathic pain. The role of nervi nervorum in the distinction between acute nociceptive and neuropathic pain. Rev Dor. 2016; 17(1):5-10. https://doi.org/10.5935/1806-0013.20160038
21. Nayak S, Sahoo AM, Chakraborti CK. Anthelmintic activity study of *Saraca indica* leaves extracts. IJABPT. 2011; 2(2):377-379.

22. Hariprasad MG, Razdan R, Yasha TC, Amrutanand ST. Thyroxine: A putative neuroprotectant in diabetes induced peripheral neuropathy in rats. J Diabetes Metab. 2015; 6(9).
23. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature. 2000; 405(6783):183-187. PMID: 10821274. https://doi.org/10.1038/35012076
24. Allchorne AJ, Broom DC, Woolf CJ. Detection of cold pain, cold allodynia, and cold hyperalgesia in freely behaving rats. Mol Pain. 2005; 1:1744-8069. https://doi.org/10.1186/1744-8069-1-36
25. Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Tabira T. Chronic stress impairs rotarod performance in rats: Implications for the depressive state. Pharmacol Biochem Behav. 2002; 71(1-2):79-84. https://doi.org/10.1016/s0091-3057(01)00636-0
26. Zhao Y, Shen F, Gong M, Jin L, Ren X, Liu K, Lu J. Lifelong treadmill training improves muscle function detected by a modified grip strength test during aging in BALB/c mice. Life sci. 2020; 251:117603. https://doi.org/10.1016/j.lfs.2020.117603
27. Nayak BP, Minaz N, Pasha K. Molsidomine ameliorates diabetic peripheral neuropathy complications in Wistar rats. Animal Model Exp Med. 2021; 4(3):243-248. Published 2021 Mar 23. https://doi.org/10.1002/ame2.12162
28. Domingo A, Ferris DP. Effects of physical guidance on short-term learning of walking on a narrow beam. Gait Posture. 2009; 30(4):464-468. https://doi.org/10.1016/j.gaitpost.2009.07.114
29. Yarim GF, Kazak F, Yarim M, Sozmen M, Genc B, Ertekin A, Gokceoglu A. Apigenin alleviates neuroinflammation in a mouse model of Parkinson's disease. Int J Neurosci. 2022:1-10. PMID: 35698811. https://doi.org/10.1080/00207454.2022.2089136
30. Rizk FH, Soliman NA, Kashef SM, Elsaadany AA. Lipoxin A4 attenuated dexamethasone-induced muscle atrophy via activation of PGC-1α/Nrf2/TFAM pathway. J Physiol Biochem. 2023; 79:107-115. https://doi.org/10.1007/s13105-022-00925-1
31. Ahmed Amar SA, Eryilmaz R, Demir H, Aykan S, Demir C. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. Aging Male. 2019 Sep; 22(3):198-206. doi: 10.1080/13685538.2018.1488955. Epub 2018 Oct 15. PMID: 30322333.
32. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-358. https://doi.org/10.1016/0003-2697(79)90738-3
33. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82(1):70-77. https://doi.org/10.1016/0003-9861(59)90090-6
34. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984; 105:121-126. PMID: 6727660. https://doi.org/10.1016/s0076-6879(84)05016-3
35. Makhlouf MMM. Histological and ultrastructural study of Aflatoxin B1 induced neurotoxicity in Sciatic nerve of adult male Albino rats. Ultrastruct Pathol. 2020; 44(1):52-60. https://doi.org/10.1080/01913123.2019.1709933
36. Turedi S, Yulug E, Alver A, Bodur A, Ince I. A morphological and biochemical evaluation of the effects of quercetin on experimental sciatic nerve damage in rats. Exp Ther Med. 2018; 15(4):3215-3224. https://doi.org/10.3892/etm.2018.5824
37. Ricci V, Ricci C, Cocco G, Gervasoni F, Donati D, Fari G, ozcakar L. Histopathology and high-resolution ultrasound imaging for peripheral nerve (injuries). J Neurol. 2022; 269(7):3663-3675. https://doi.org/10.1007/s00415-022-10988-1
38. Mansueto G, Benincasa G, Capasso E, Graziano V, Russo M, Niola M, Napoli C, Buccelli C. Autoptic findings of Sudden Cardiac Death (SCD) in patients with Arrhythmogenic Ventricular Cardiomiopathy (AVC) from left ventricle and biventricular involvement. Pathol Res Pract. 2020; 216(12):153269. https://doi.org/10.1016/j.prp.2020.153269
39. Jain KK. Drug-induced peripheral neuropathies. Drug Induced Neurological Disorders. Springer Nature. 2021: pp. 437-467. https://doi.org/10.1007/978-3-030-73503-6_28
40. Singh R, Rao HK, Singh TG. Neuropathic pain in diabetes mellitus: Challenges and future trends. Obes Med. 2020; 18:100215. https://doi.org/10.1016/j.obmed.2020.100215
41. Galiero R, Caturano A, Vetrano E, Beccia D, Brin C, *et al*. Peripheral neuropathy in diabetes mellitus: Pathogenetic mechanisms and diagnostic options. Int J Mol Sci. 2023; 24(4):3554. https://doi.org/10.3390/ijms24043554
42. Rasekar V, Shahi S. Medical application of Ashok tree (*Saraca asoca*): A review. Int J Health Sci. 2022; 6(S2). https://doi.org/10.53730/ijhs.v6ns2.7262
43. Thilagam E, Chidambaram K, Raviteja C, Vahana T, Vasudevan P, Garrido G. Anti-hyperglycemic and hypolipidemic effects of *Saraca asoca* (Roxb.) Wild. flowers in alloxan-treated diabetic rats. J Pharm Pharmacogn Res. 2021; 9(1):58-68. https://doi.org/10.56499/jppres20.894_9.1.58
44. Maurotti S, Pujia R, Galluccio A, Nucera S, Musolino V, Mare R, *et al*. Preventing muscle wasting: Pro-insulin C-peptide prevents loss in muscle mass in streptozotocin-diabetic rats. J Cachexia Sarcopenia Muscle. 2023; 14(2):1117-1129. https://doi.org/10.1002/jcsm.13210
45. Grover A, Sharma K, Gautam S, Gautam S, Gulati M, Singh SK. Diabetes and its complications: Therapies available, anticipated and aspired. Curr Diabetes Rev. 2021; 17(4):397-420. https://doi.org/10.2174/1573399816666201103144231
46. Sahayam GP, Palani S. Association of inflammatory marker, glycosylated hemoglobin, circulating lipids with microvascular complications and glycemic control of Type

2 diabetes mellitus in South Indian population. Rom J Diabetes Nutr Metab Dis. 2021; 28(3):261-267.
47. Liyew WA. Clinical presentations of lumbar disc degeneration and lumbosacral nerve lesions. Int J Rheumatol. 2020. https://doi.org/10.1155/2020/2919625
48. Vague P, Dufayet D, Coste T, Moriscot C, Jannot MF, Raccah D. Association of diabetic neuropathy with Na/K ATPase gene polymorphism. Diabetologia. 1997; 40(5):506-511. PMID: 9165217. https://doi.org/10.1007/s001250050708
49. Mbiantcha M, Khalid R, Atsamo DA, Njoku IS, Mehreen A, Ateufack G, Hamza D, Nana WY, Naeem RU, Izhar A. Anti-hypernociceptive effects of methanol extract of *Boswellia dalzielii* on STZ-induced diabetic neuropathic pain. Adv Tradit Med. 2020; 20:405-417. https://doi.org/10.1007/s13596-019-00411-y
50. Liu J, Nie Y, Chaudhry M, Bai F, Chuang J, Sodhi K, Shapiro JI. The redox-sensitive Na/K-ATPase signaling in uremic cardiomyopathy. Int J Mol Sci. 2020; 21(4):1256. https://doi.org/10.3390/ijms21041256
51. Bigorra MS, Rochina IJ, Cauli O. Differences and similarities in neuropathy in Type 1 and 2 diabetes: A systematic review. J Pers Med. 2021; 11(3):230. https://doi.org/10.3390/jpm11030230
52. Bolajoko EB, Akinosun OM, Khine AA. Hyperglycemia-induced oxidative stress in the development of diabetic foot ulcers. Diabetes. 2nd Edition. Academic Press. 2020: pp. 35-48. https://doi.org/10.1016/b978-0-12-815776-3.00004-8
53. Hussain G, Wang J, Rasul A, Anwar H, Qasim M, Zafar S, *et al*. Current status of therapeutic approaches against peripheral nerve injuries: A detailed story from injury to recovery. Int J Biol Sci. 2020; 16(1):116-134. https://doi.org/10.7150/ijbs.35653
54. Park CH, Min KB, Min JY, Kim DH, Seo KM, Kim DK. Strong association of Type 2 diabetes with degenerative lumbar spine disorders. Sci Rep. 2021; 11(1):16472. https://doi.org/10.1038/s41598-021-95626-y
55. Yam MF, Loh YC, Oo CW, Basir R. Overview of neurological mechanism of pain profile used for animal "pain-like" behavioral study with proposed analgesic pathways. Int J Mol Sci. 2020; 21(12):4355. https://doi.org/10.3390/ijms21124355
56. Chen SM, Wang MH, Soung HS, Tseng HC, Fang CH, Lin YW, Yang CC, Tsai CC. Neuroprotective effect of 1-theanine in a rat model of chronic constriction injury of sciatic nerve-induced neuropathic pain. J Formos Med Assoc. 2022; 121(4):802-814. https://doi.org/10.1016/j.jfma.2021.08.023
57. Dash JR, Pattnaik G, Ghosh G, Rath G, Kar B. Protective effect of epicatechin in diabetic-induced peripheral neuropathy: A review. J Appl Pharm Sci. 2023; 13(1):56-63. https://doi.org/10.7324/japs.2023.130105-1
58. Huang J, Huang A, Poplawski A, DiPino Jr F, Traugh JA, Ling J. PAK2 activated by Cdc42 and caspase 3 mediates different cellular responses to oxidative stress-induced apoptosis. Biochim Biophys Acta Mol Cell Res. 2020; 1867(4):118645. https://doi.org/10.1016/j.bbamcr.2020.118645