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RESEARCH ARTICLE

Examining the *Saraca asoca* (*Caesalpinaceae*) Methanolic Leaf Extract for its Potential to Protect Peripheral Neuropathy Caused by Cisplatin in Albino Rats

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Abstract

Background: Peripheral neuropathy, a debilitating side effect of platinum-based chemotherapy like cisplatin, manifests as severe neuropathic pain and functional impairments. *Saraca asoca*, a medicinal plant recognised for its neuroprotective properties, warrants exploration for its efficacy in mitigating Cisplatin-Induced Peripheral Neuropathy (CIPN). **Aim:** To evaluate the neuroprotective potential of *S. asoca's* methanolic leaf extract against CIPN in albino Wistar rats and identify bioactive phytoconstituents responsible for these effects. **Methods:** twenty-four male Wister rats were divided into four groups: normal control, disease control (cisplatin 2mg/kg), and two treatment groups receiving *S. asoca* methanolic leaf extract (200 mg/kg and 400 mg/kg) alongside cisplatin for 28 days. Behavioural assessments (rotarod, grip strength, hanging wire tests, and others), haematological parameters (C-reactive protein), oxidative stress markers (malondialdehyde, catalase, and glutathione), and histopathological analysis of the sciatic nerve were conducted. Phytochemical screening and chromatographic techniques (TLC, HPTLC) were employed to characterise the extract. **Results:** *Saraca asoca* treatment ameliorated cisplatin-induced behavioural deficits, restored oxidative stress parameters, and reduced C-reactive protein levels. Histopathological analysis showed improved nerve architecture with reduced inflammatory infiltration and myelin degeneration in treated groups, especially at 400mg/kg. The phytochemical analysis confirmed the presence of neuroprotective constituents such as quercetin dehydrate. **Conclusion:** The methanolic leaf extract of *S. asoca* demonstrated a significant neuroprotective effect against CIPN. CIPN by modulating oxidative stress, inflammation, and histopathological integrity. These findings highlight the potential of *S. asoca* as a complementary therapy for CIPN, warranting further investigation into its underlying mechanisms and clinical application.

Major Findings: *Saraca asoca* leaf extract at 400 mg/kg significantly mitigated CIPN in rats by enhancing antioxidant defenses, reducing inflammation, and preserving nerve structure—resulting in improved behavioral performance and weight preservation. Quercetin dehydrate was identified as a key active compound contributing to these effects.

Keywords: Cisplatin-induced Peripheral Neuropathy, Neuroprotection, Oxidative Stress, Phytochemicals, *Saraca asoca*

Abbreviations: CIPN: Cisplatin-Induced Peripheral Neuropathy; CAT: Catalase Activity; CCK2: Cholecystokinin Receptor; DTNB: Disodium Hydrogen Phosphate, 5, 5′-Dithiobis - (2-Nitrobenzoic Acid); GSH: glutathione; HPLC: High-Performance Liquid Chromatography HPTLC: High-Performance Thin Layer Chromatography; H and E: Hematoxylin and Eosin MsT: Masson trichrome; MDA: Malondialdehyde; NADH: Nicotinamide Adenine Dinucleotide; ROS: Reactive Oxygen Species; RVM: Rostroventromedial medulla; SA: *Saraca asoca*; TLC: Thin Layer Chromatography; TBA: Thiobarbituric Acid.

1. Introduction

Peripheral neuropathy is a condition characterised by symptoms arising from nerve damage outside the brain and spinal cord, known as peripheral nerves. These nerves transmit sensations to the brain, control arm and leg movement, and regulate bladder and bowel functions[1]. The contemporary way of life is

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often linked to health issues such as diabetes, cancer, peripheral neuropathy, and other lifestyle choices that include inactivity[2]. As the number of cancer patients rises, families, communities, and healthcare systems are under strain. The person seems unprepared because high-quality diagnosis and therapy are not readily available in many low- and middle-income nations[3].

Globally, Cancer was the second most prevalent cause of mortality, accounting for 9.6 million fatalities. Breast, colorectal, lung, thyroid, and stomach cancers are more common in women than in males, as are lung, prostate, colorectal, stomach, and liver cancers[4]. The International Agency for Research on Cancer lists several variables as cancer-causing agents and states that as people age, their chance of developing a particular disease increases and less effective cellular repair mechanisms, including tobacco use, air pollution, bad eating habits, alcohol use, and inactivity[5]. CIPN caused by chemotherapy, a potentially serious side effect of cancer treatment, can result in peripheral nerve injury, a common side effect of various cancer treatments[6]. CIPN can result in excruciating discomfort and interfere with everyday tasks including walking, writing, and coin-picking[7]. It can last for weeks, months, or years post-treatment, potentially leading to serious issues like heart rate and blood pressure changes, falls, breathing difficulties, paralysis, or organ failure. Treatment for peripheral neuropathy requires an understanding of its aetiology, particularly in individuals undergoing CIPN.

Ayurveda, a traditional Indian treatment, uses leaves and extracts from nearby trees and plants to cure various ailments, including unknown health benefits. Because of its therapeutic qualities, *S. asoca* is considered a wonderful remedy in *Ayurveda*. It is mostly used to treat problems specific to women's illnesses in Nepal, India, and Sri Lanka[8,9]. In Indian forests, the tiny, evergreen *S. asoca* tree grows close to streams of water, and subcontinent forests. It is planted in gardens as an ornamental plant. Its compound leaves are purplish when young and green when mature, and its fragrant, scarlet orange flowers are borne in dense clusters. Its stem bark is used to prepare *Ashokarishta*, an *Ayurvedic* tonic for menstruation-related problems[10].

A study revealed that the flavonoids from *S. asoca* have antioxidant properties that enhance their anti-carcinogenic properties by reducing carcinogen bioavailability and preventing liver biotransformation and have potential chemo-preventive properties against 2-stage skin carcinogenesis[11]. Along with a broad spectrum of ethnobotanical applications and pharmacological characteristics, such as anti-cancer, action against menorrhagia that is antimicrobial, anti-implantation, anti-tumour, anti-oxytocic, uterotonic, anti-progestational, and anti-estrogenic, Cardioprotective, hepatoprotective, CNS depressant activity, anti-mutagenic and renoprotective activities, analgesic, anti-ulcer, anti-pyretic, anti-helminthic, genetic studies, antifungal activity, antibacterial activity, immunomodulatory property, spasmogenic, anti-inflammatory, anti-arthritic, anti-estrogenic, and hypoglycemic, anti-diarrhoea, menstrual cramps, stomach pain, and anti-depressant medications[12–16].

The bark cleanses the blood and gives women's complexion a lovely, healthy shine. Boil the leaves in water to remove greasy and dead skin, then eat them when cooled. On the skin, apply a paste prepared from the bark or crushed leaves of the tree. Use a powder or pill twice a day after meals as a homemade treatment for gas and cramps[17,18].

Previously, plant components were utilised directly for therapy, but now active principles are extracted and synthesised using advanced technology[19]. Chemical structures created from these phytoconstituents can serve as models for creating novel synthetic medicines[20]. Identifying phytoconstituents in plant material can assist in anticipating the plant's potential pharmacological action. Plant characterisation and assessment, as well as their phytoconstituents, can provide data to support medicinal claims against various maladies. Innovative techniques like Thin Layer Chromatography (TLC), High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC) are beneficial for detecting phytoconstituents both subjectively and quantitatively[21]. However, when these techniques are unavailable or prohibitive, conventional phytochemical assays, which are economical, simple, and need fewer resources, remain the appropriate alternative for early phytochemical screening[22].

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[23]. Gallic acid, leucocyanidin, Lucocyanidin, palmitic, stearic, linolenic, linoleic,

cyanidin-3, 5-diglucoside, pelargonidin-3, and quercetin are among the compounds that have been identified[24]. The ethanol extract of *S. asoca* leaves used in the phytochemical screening test contained alkaloids, saponins, phenols, tannins, and flavonoids[25]. Alkaloids, flavonoids, terpenoids, and steroids are among the secondary metabolite chemicals in *S. asoca* leaf methanol extract[26]. Test samples consist of fractions, crude extracts, and pure compounds derived from natural components[27].

This study aims to separate the methanolic extraction of *S. asoca* leaves to follow up on previous findings on oral administration. Using TLC, Column chromatography, HPTLC, and a preliminary test to validate phytoconstituents, it also sought to evaluate the peripheral neuropathy activity in vivo versus peripheral neuropathy produced by Cisplatin and flavonoid analysis. The findings of this research will assist in identifying the existence of antioxidant activity linked to *S. asoca* for health benefits and prevention of peripheral neuropathy.

2. Materials and Methods

2.1 Chemicals and Reagents

Cisplatin (West-Coast Pharmaceutical Works Pvt. Ltd, India), 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-(2-Nitrobenzoic Acid), 5,5′-Dithiobis, Methanol, Normal Saline, Malondialdehyde, glutathione, and Catalase were measured using a commercially available kit were obtained from vasa scientific Pvt. Ltd, Bangalore, India. The investigation utilised analytical-grade medicines and chemical reagents.

2.2 Animals

The study involved adult male Wistar rats, weighing 200 ± 20 g, obtained from a licensed breeder (Biogen Laboratory Pvt.Ltd.), Bangalore. Before the trial began, the rats were given seven days to become used to their new surroundings. They were kept at 24°C±1°C, with a 45–55 % relative humidity and a 12–12 h light/dark cycle. They were housed in sterile propylene cages with sterile husks that were replaced every day. Throughout the experimental treatment, the animals were provided with an unlimited supply of water and a regular pellet chow meal. Every experiment was conducted from 9:00 to 17:00. The Institutional Animal Ethics Committee (IAEC) of KLE College of Pharmacy, Bangalore, granted authorization for the experimental approach, which was conducted by the guidelines of the Committee for Control and Supervision of Animal Experiments (CPCSEA) (IAEC/01/HP/202).

2.2.1 Preparation of Plant Material

In October 2021, fresh *S. asoca* leaves were procured from Bangalore's Indian Institute of Horticultural Research (IIHR). Dr. P. E. Rajasekharan, Principal Scientist and Nodal Officer, GSC Division of Plant Genetic Resource, has confirmed the plant.

2.2.2 Extraction of Leaves

The *S. asoca* leaves were chopped into little pieces using a wood grinder after being cleaned three times under running water and shade-dried. Dried samples of the *S. asoca* were crushed into a coarse powder. A previously published procedure[28] was used for the extraction process, and a 3000 ml Soxhlet apparatus was filled with around 250 g of coarsely ground material and extracted using methanol as a solvent for 3 consecutive days using a continuously running method and the boiling point was maintained at 64.7°C. After the extraction, evaporation was done using a Rota evaporator, and the solvent was recovered and reused.

2.2.3 Dose Section of S. asoca Methanolic Leaf Extract

The doses of 200mg/kg and 400mg/kg were chosen based on findings from Varghese et al., who investigated the potential anticancer activity of *S. asoca* extracts in mice[29]. Their study demonstrated that these dose ranges were effective and safe in prior studies investing antitumor activity, ensuring the relevance for evaluating neuroprotective effects in the current study.

2.2.4 Phytochemical Screening of S. asoca Methanolic Leaf Extract

Using the following technique, a tiny sample of *S. asoca* methanolic leaf extract was examined for several phytoconstituents: Concentrated sulphuric acid (H_2SO_4) was utilized in the steroid test, sodium hydroxide (NaOH) was used in the tannin test, and ferric chloride was used in the flavonoid test. Dragendroff and Mayer's reagents were employed in the alkaloids test, whereas foam reagent was utilized in the Saponin test, Chloroform and sulphuric acid were employed in the terpenoids test, acetic acid and chloroform in the glycoside test, and Molisch's test and Barfoed's test reagent in the carbohydrate test[30,31].

2.2.5 Thin Layer Chromatography (TLC)

The study used 10x10 cm reactivated TLC silica gel plates to separate quercetin flavonoids from *S. asoca* methanolic leaf extract. The solvent system used was toluene, ethyl acetate, and methanol (80:10:10) fractions were mixed and repeated column chromatography. TLC silica gel G was used to separate quercetin dihydrate from the methanolic extract of *S. asoca* leaf.

The plates were then placed within a UV chamber saturated with iodine vapours to observe the colour of the spot. The developed plate was sprayed with 5% ethanolic ferric chloride to observe the colour of a spot in both the sample and quercetin dihydrate. An isolated sample's Rf values were computed and compared to the corresponding standard. Mixed fractions were concentrated and preserved for further analysis[32].

2.2.6 Column Chromatography (CC) Analysis

As previously noted, toluene, ethanol, and methanol (80:10:10) were used as the mobile phases in the preparation of the column. The sample's filter-dried methanolic extract was combined with silica gel G and the solvent. The extract containing silica was scraped off and placed into butter paper before being deposited onto the top of the packed column after being dried in a water bath. Following the loading of the sample, the solvent was added, and the setup for the fractional separation was monitored. The fractions were collected in test tubes, for every fragment, TLC was used to verify the purity of the fractions, and the formula given was used to calculate the Rf values[33].

Retention factor = Distance travelled by the solute/ Distance travelled by the solvent

After allowing the solvent to evaporate, the fractions' dry weight was recorded.

3. High-Performance Thin Layer Chromatography Analysis (HPTLC)

3.1 Preparation of Standards and Sample Solution

3 ml of methanol was used to dissolve 5 mg of *S. asoca* leaf extract. After five minutes of sonication, using the same solvent, the final volume was raised to 5 ml, producing a stock solution with a concentration of 1 mg/ml. 10 ml of methanol was used to extract the 0.5g air-dried sample. The extracts underwent filtration, concentration, and a final volume increase to 10 ml with methanol to produce a stock solution containing 50 mg/ml before to HPTLC examination[20,21].

3.1.1 Experimental Design

After that, four groups of six rats each were randomly allocated to the twenty-four healthy rats. After the rats had been acclimated for seven days, they were randomly assigned to the following groups: Group I is a normal control; Group II is a disease control. The control group was given only cisplatin 2mg per kg body weight (twice weekly), and to prevent nephrotoxicity induced by the cisplatin, 2 ml of saline solution was administered orally before Intraperitoneal administration of Cisplatin[34]. Group III is a low dose of *S. asoca* leaf extract (200 mg/kg of body weight taken orally) + Cisplatin; Group IV is a high dosage of *S. asoca* leaf extract (400 mg/kg of body weight taken orally) + Cisplatin, respectively.

After completion of 28 days, rats were investigated for the effect of a methanolic leaf extract of *S. asoca* in rat models of peripheral neuropathy by measurement of behavioural, biochemical, and histopathological parameters. Ultimately, sciatic nerves were promptly detached from rats after they were sacrificed under deep anaesthesia. A section of the sciatic nerve was retained by preparing a tissue homogenate in 0.1 M Tris-HCL buffer (pH 7.4) for a biochemical estimate and a formalin solution for histological assessment.

The remaining nerve tissue was maintained at -20 °C for further analysis.

3.1.2 Evaluation by Behavioral Parameters

The study examined the neuroprotective properties of *Saraca asoca* methanolic leaf extract through various behavioural tests, including body weight, thermal hyperalgesia, cold hyperalgesia, Rota-rod, grip strength, narrow beam walk, and hanging wire test, after 4 weeks of treatment.

3.1.3 Thermal Hyperalgesia

Thermal hyperalgesia was evaluated using the tail flip test, which comprised immersing the tail in hot water (46ºC) and observing for indications of resistance or retreat, in compliance with the previously defined protocol. To prevent causing harm to tissue, a 12-second cut-off time was used. Shorter tail retraction times suggest increased pain sensitivity. The tail was thoroughly dried after recording each reaction[35].

3.1.4 Cold Hyperalgesia

Following the previously established method, the rats' tails were immersed in a beaker of 4°C cold water, and the assessment of cold hyperalgesia was based on the observation of resistance or retreat. Flicking reactions or tail withdrawals from cold water were observed during the 10-second cut-off period. Cold hyperalgesia can be identified by a reduction in tail retract time[36].

3.1.5 Rota-Rod Performance

The Rota-rod test was conducted on rats to evaluate their motor coordination. Rats in the experiment were able to remain on the rod for five minutes after it was modified. The rat was placed on the rod, which rotates at a pace of 25 revolutions per minute, for three to four hours throughout the examination. Every rat's maximum amount of time spent on the Rota rod was noted[37].

3.1.6 Grip Strength Test

Rats were used to test grip strength using the method previously mentioned Bioseb's method, the Grip Strength Test device evaluates muscle strength[38]. Rats are pulled backwards in the horizontal plane while being held by their tails. To collect measurements, each rat was suspended by its tail, which allowed for a nearly horizontal alignment over the grid of the grip strength meter. The rat was given a gentle nudge at the base of its tail in line with the sensor's axis until it let go of the grid. The force of the rat was expressed in Newtons or kg[39].

3.1.7 Narrow Beam Walk Test

A short, flat, fixed wooden plank that measured two feet long by one centimetre in width and 100 centimetres above the ground could be crossed by trained animals. The evaluation was conducted using the previously mentioned methodology[40]. Measurements were made on the amount of time needed to traverse the beam from one end to the other as well as foot slip mistakes. The study's conclusion (day 28) is the impact of the *S. asoca* leaf extract time needed to traverse the beam from one end to the other[41].

3.1.8 Hanging Wire Test

The hanging-wire test was used to test neuromuscular strength in rats. Every rat was set up on a cage's wire lid, and the time it took for the rat to remain still until at least two of its limbs were on the lid was recorded. The longest latency was recorded. Previously trained animals were permitted to hang onto a horizontal grid, which was turned upside down for upside-down hanging. The impact of *S. asoca* on the Cisplatin-induced hanging test was evaluated at the end of the trial[42].

3.1.9 Determination of C-Reactive Protein (CRP)

A plasma protein that is phylogenetically highly conserved and involved in the systemic response to inflammation is C-reactive protein (CRP). A retro-orbital sinus puncture was performed on day 30, before the rats' sacrifice, to draw blood and measure the C-reactive protein level. A CBC analyzer (Rohana Veterinary Diagnostic lab) was used to get the C-reactive protein values from each blood sample after it had been spun for 15 minutes[43].

3.2 Oxidative Stress Parameters

Sciatic nerves were isolated and homogenized using a probe homogenizer in ice-cold phosphate buffer (0.1M, pH 7.4) after the experiment. The protein content in tissue homogenate was measured using a Folin phenol reagent[44]. Malondialdehyde (MDA) was used

to measure lipid peroxidation[45]. Reduced glutathione (GSH) levels were used to determine tissue sulfhydryl groups[46]. Catalase activity was measured as the amount of hydrogen peroxide broken down in a minute[47]. Furthermore, the MDA, GSH, and catalase activity was determined using the following formula:

- Concentration of MDA = absorbance (test)-absorbance (blank)/1.56 × 10.00.000
- Concentration of GSH = (absorbance of test-absorbance of blank) × 2 × 3.1 × df / (6.22) (0.05)
- Concentration of CAT = 2.3At × initialx1.63x10-3 /final

3.3 Histopathological Evaluation

The sciatic nerve was carefully separated and treated in a 10% formalin solution following the collection of blood samples. Histopathology examinations were performed using standard procedures. A section was embedded in paraffin and preserved in a 10% glutaraldehyde solution. A rotary microtome was used to create serial tissue slices, which were then processed with Masson's trichrome and Mayer's hematoxylin and eosin stain[48].

3.4 Statistical Analyses

The Mean ± SEM was used to evaluate and display the fundamental data, with six members in each group. Dunnett's multiple comparison tests were employed to investigate any significant differences ($p<0.05$) between the groups after the data were compared using a one-way ANOVA. GraphPad Prism version 8.0.2 was utilized to examine this data.

4. Results

4.1 *Saraca asoca* Methanolic Leaf Extract using HPTLC

The methanolic extract of dried *Saraca asoca* leaf extract yielded quercetin dihydrate thin layer chromatograms at 366 nm. Quercetin dihydrate bands were identified and confirmed in the sample chromatogram, with the retention time of 38.806 and 38.616, which nearly coincide with the standard retention time of quercetin dihydrate Figure 1.

4.2 Impact of *Saraca asoca* Methanolic Leaf Administration on Body Weight

The disease control group experienced a significant weight loss, which was mitigated by the administration of *Saraca asoca* at doses of 200mg/kg and 400mg/kg shown in Figure 2. A substantial decrease in body weight was observed from 223±1.8g to 100±4.7g ($p<0.001$). Additionally, 400mg/kg *S. asoca* administration significantly increased body weight, reaching 177±1.7g ($p<0.001$) compared to the disease control group.

4.3 Impact of *S. asoca* Leaf Treatment on Behaviour Parameters

The group under disease control considerably decreased ($p<0.001$) in reaction delay and tail withdrawal delay in contrast to the normal group in both hot and cold-water tail immersion tests. In contrast to the disease control group, a four-week *S. asoca* therapy at all examined dosage levels significantly offset the decrease in latency. The high dose of *Saraca asoca* has increased comparatively in the rota-rod, hanging wire, grip strength, and narrow beam walk behaviour 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 shown in Figure 3.

4.4 Impact of *Saraca asoca* Leaf Therapy on Rat's Haematology

The haematological results, including C-reactive protein (CRP), are shown in Table 1. Except for the disease control group, all groups receive *S. asoca* therapy for 28 days with 2ml saline orally. However, the high-dose observations (400 mg/kg) ($p<0.001$) group show a considerable improvement over the disease control group.

4.5 Impact of *S. asoca* Leaf Treatment on Oxidative Stress Indicators

In contrast to rats in the disease control group, the illness exhibited a significant rise in oxidative stress in Sciatic nerve homogenate, as demonstrated by a significant increase in Malondialdehyde (MDA) levels and a decrease in glutathione (GSH) and catalase concentrations. *Saraca asoca* therapy at 200mg/kg and 400mg/kg significantly reduced MDA levels ($p<0.001$,

respectively) contrasted to the disease control group. GSH levels increased significantly after *Saraca asoca* treatment at dosages of 200mg/kg ($p<0.05$) and 400mg/kg ($p<0.01$). Additionally, after receiving *Saraca asoca* treatment, the concentration of catalase rose considerably in comparison to the disease control group depicted in Table 2.

4.6 Impact of *Saraca asoca* Therapy on the Histology of the Sciatic Nerve

Scientific photomicrographs using light were investigated to evaluate the impact of various dosages of *S. asoca* leaf extract demonstrated in Figures 4 and 5. 1A. Normal histology given the longitudinal sections of the sciatic nerve, the Nerve Fibres (NF) are bundled, parallel, wavy, uninterrupted, and in a bundle with SWC (Schwann cell) nuclei Hematoxylin and eosin (HandE) x 300. 1B. The myelin sheath (MY) surrounding the axonal appendages is in normal appearance, thin endoneurial collage (ENC) enveloping the myelin sheath in normal histological appearance, and Connective Tissue (CT) surrounding axonal appendages Masson trichrome (MsT) x 300. 1B. control group given the transversal slice demonstrating the endoneurium's decreased nerve fibre density. Demonstrates increased vascular proliferation and myelin degeneration and decreased Schwann cells in Cisplatin-administrated rats. The black arrow indicates that fibres were degenerated and misarranged with large cracks, and the red arrow indicates myelin vacuolization. 2B, sciatic nerve neuroaxonal degeneration (also known as "spheroids") after treatment with the diketone chemical N-hexane. Axons with pale staining and varying sizes, together with thin myelin sheaths, are characteristic of affected nerve fibres. An arrow is used to illustrate this. Subcellular abnormalities found in distended axons include cytoskeletal filament accumulations, tubulovesicular profiles, granular/membranous entities, and deformed mitochondria (which do not occur in light microscopic preparations) stained HandE x 200. 2C, Retin bodies are subperineurial collections of elongated, loosely whorled, mucin-rich, and cell-poor

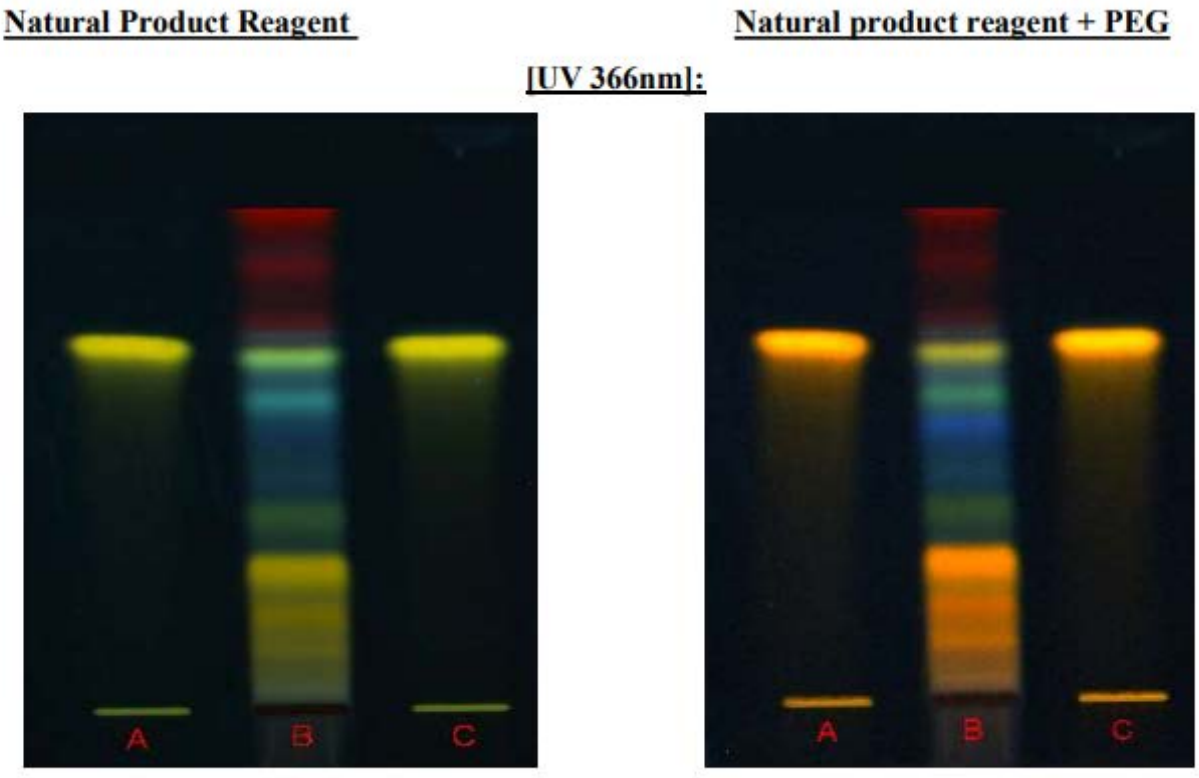


Figure 1. Fingerprint of plant extract High-performance thin layer liquid Chromatograms (HPTLC) obtained at 366nm. Mobile Phase**:** Toluene: Ethyl Acetate: Methanol [80:10:10] **(A).** Quercetin Dihydrate Reference standard, **(B).** *S. asoca* leaf extract-methanolic; **(C).** Quercetin Dihydrate Ref Std/NR/Lot No: T2K083.

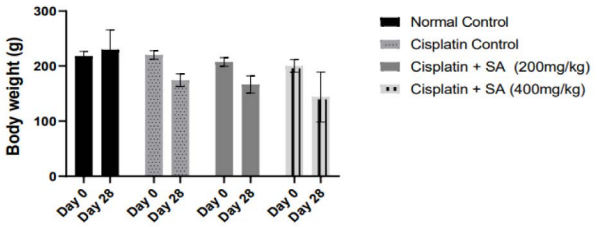


Figure 2. *S. asoca* methanolic leaf treatment on CIPN in rats: body weight on the injection day (Cisplatin) after 0 days and 28 days. Mean± SEM (n = 6) was used to express the data. ### p<0.001vs.normal control; ***p<0.001 vs. diseased control.

connective tissue (arrows in A and B). These results are frequently seen in sensorimotor nerves, and nerve compression may be the cause. Both frequency and intensity might rise if the test object is administered in a way that causes prolonged recumbency (as an indirect consequence). 2D, Spinal nerves (N) and their branches are surrounded by a distribution of arteries and veins. The sciatic nerve has diffuse Schwann cell nuclei and a large amount of eosinophilic myelin (arrows), which are distinctive characteristics of the most often used peripheral nervous system sample in animal investigations. E (epineurium) stained with HandE x 300.1C. The distribution of arteries (A) and veins is correlated with the spinal nerves (N) and

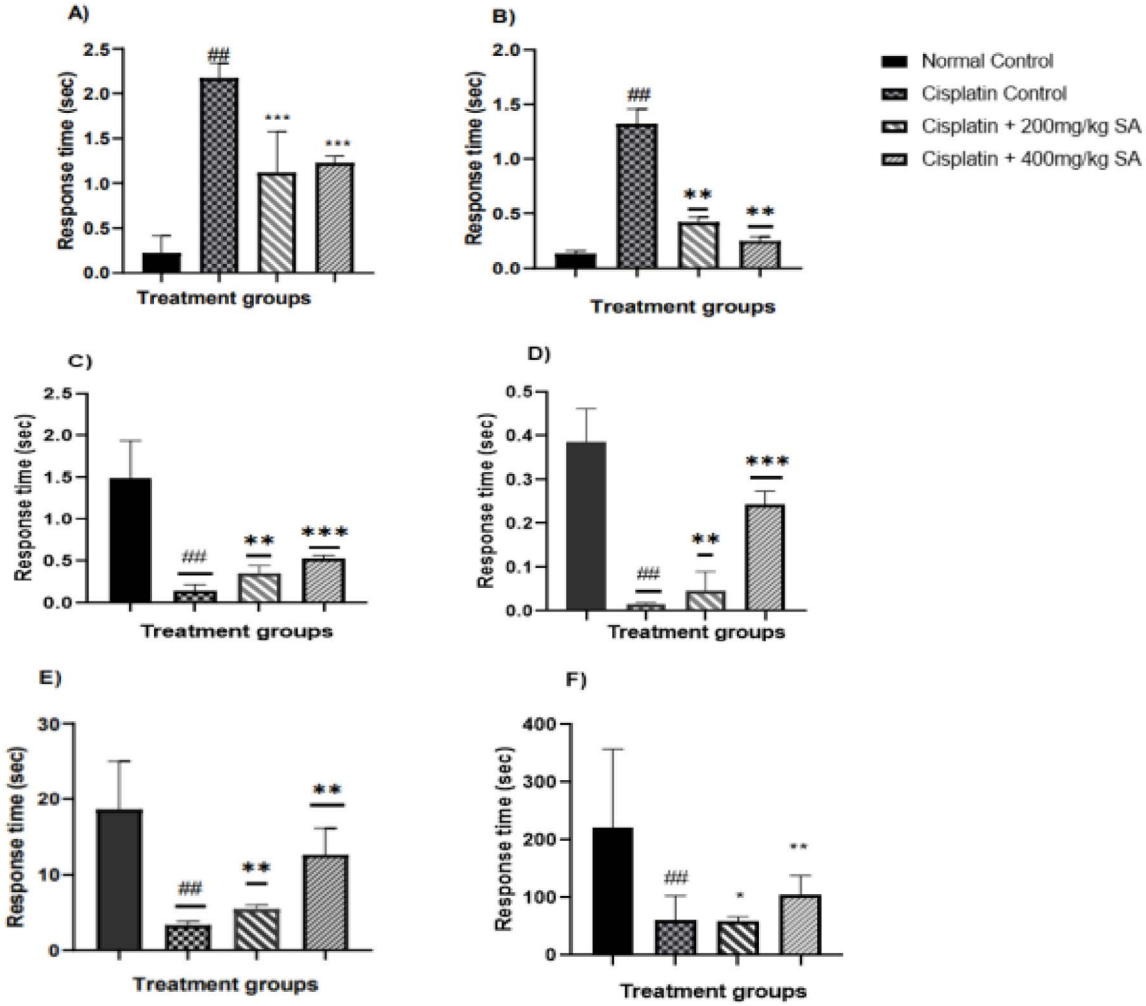


Figure 3. *S. asoca* methanolic leave treatment on CIPN in rats: **(A).** Thermal hyperalgesia; **(B).** Cold hyperalgesia; **(C).** The rotarod test; **(D).** The hanging wire test; **(E).** Grip Strength test; **(F).** Narrow beam walk test. Mean± SEM (n = 6) was used to express the data. ###p<0.001vs. normal control; ***p<0.001 vs. diseased control.

their branches. E is for epineurium, proliferation, and regeneration of SWC HandE x 200. 2C, spinal nerve root exhibiting primary demyelination. Affected nerve fibres exhibit extensive myelin loss and intramyelinic fluid collection, resulting in vast gaps surrounding their core axons (arrow). Numerous plumps and foamy macrophages can be seen in the interstitial spaces between nerve fibres. Reactive SWC and reduced image of endoneurial collagen (ENC), axoplasmic vacuole (VC) surrounding myelin (MY) MsT x 100. 1D, interruption in nerve fibre continuity, oedema (EDM), diffuse vacuole (V), pronounced hyperthermia in SWC HandE x 300. 2D, Degeneration of the myelin sheath (MD), the number of mass cells that attract attention (MC), obvious collagen fibre increase, degenerative nerve bundles and disruption of the myelin sheath, the presence of mass cells and specialized macrophage MsT x 200.

Table 1. Effects of *S. asoca* methanolic leaf extract on CRP parameters in CIPN rat model. C-reactive protein in rats was measured in mg/l, after the last dose of administration of *S. asoca* methanolic leaf extract. Mean± SEM (n = 6) was used to express the data

Sl.no	Groups	CRP (mg/dL)
1	Normal control	0.67± 0.082
2	Disease control	0.69± 0.084
3	Cisplatin + *SA* (200mg /kg)	0.63±0.053
4	Cisplatin + *SA* (400mg /kg)	0.67± 0.82

5. Discussion

In this work, we have shown that in a rat model of preventative CIPN, *Saraca asoca* leaf extract ingested once a day over 28 days helped alleviate symptoms of neuropathy.

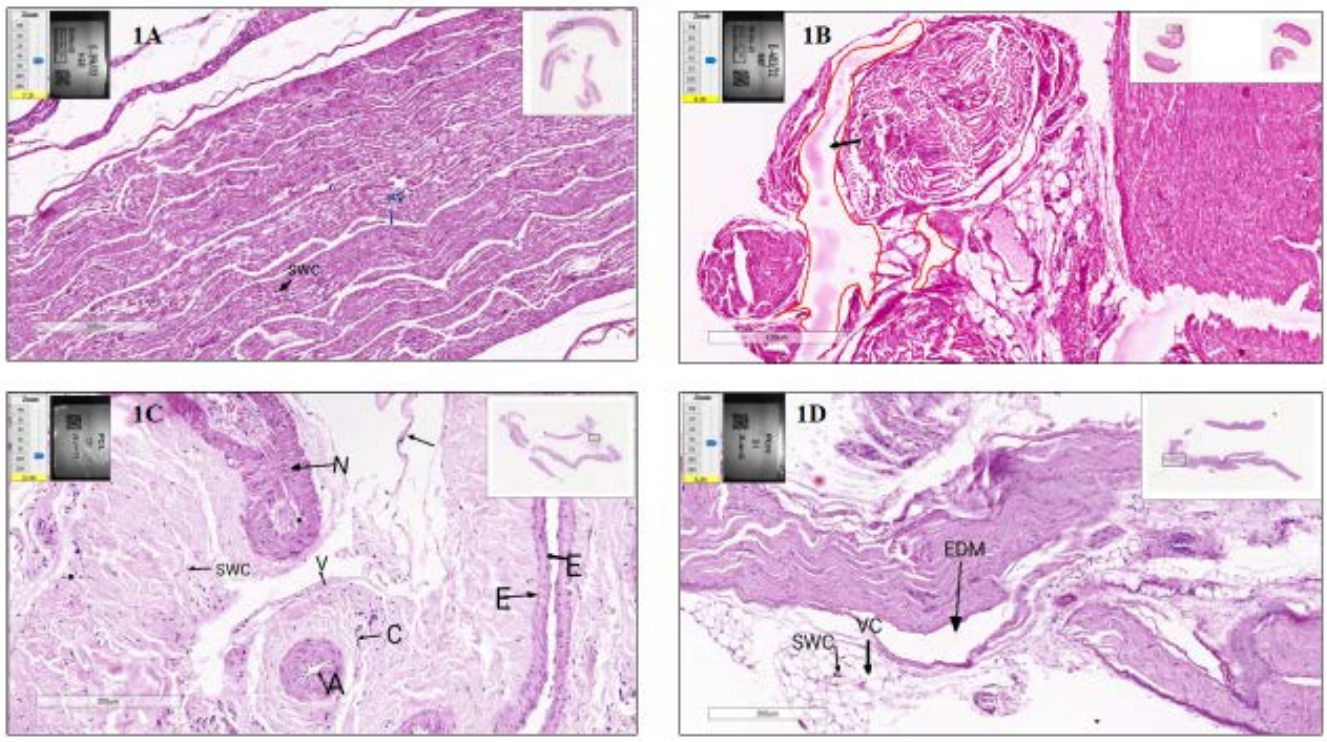


Figure 4. Light photomicrographs of the sciatic nerve were examined to assess the effect of different doses of *S. asoca* methanolic leaf extract- H and E staining. 1A, Normal Control: showing normal histology given the longitudinal sections of the sciatic nerve, the nerve fibres (NF) are bundled, parallel, wavy, uninterrupted, and in a bundle with SWC (Schwann cell). 1B, Disease Control: displaying degenerative abnormalities in the neuroaxis, such as axonal swelling and myelin loss 1C, 1D *S. asoca* (200 mg/kg and 400 mg/kg): displaying typical histopathology, limited inflammatory cell penetration in the myelin sheath, the distribution of arteries and veins is correlated with the spinal nerves. X is represented by the scale bar at 200 µm, 300 µm, and 400 µm.

Table 2. Catalase, MDA, and GSH for *S. asoca* leaf therapy on CIPN. Analysis of CAT, MDA, and GSH for different treatment groups and control groups was found to be significant (p<0.001)

Groups	Catalase (protein U/mg)	MDA (protein, nmol/mg)	GSH (protein, nmol/mg)
Normal control	0.10925±0.022	0.1235±0.03	0.06525±0.020
Disease control	0.084±0.026	0.1505±0.04	0.06775±0.025
Cisplatin + SA (200mg/kg)	0.13775±0.025	0.12775±0.03	0.06725±0.021
Cisplatin+ SA (400mg/kg)	0.11075±0.42	0.12675±004	0.0665±0.021

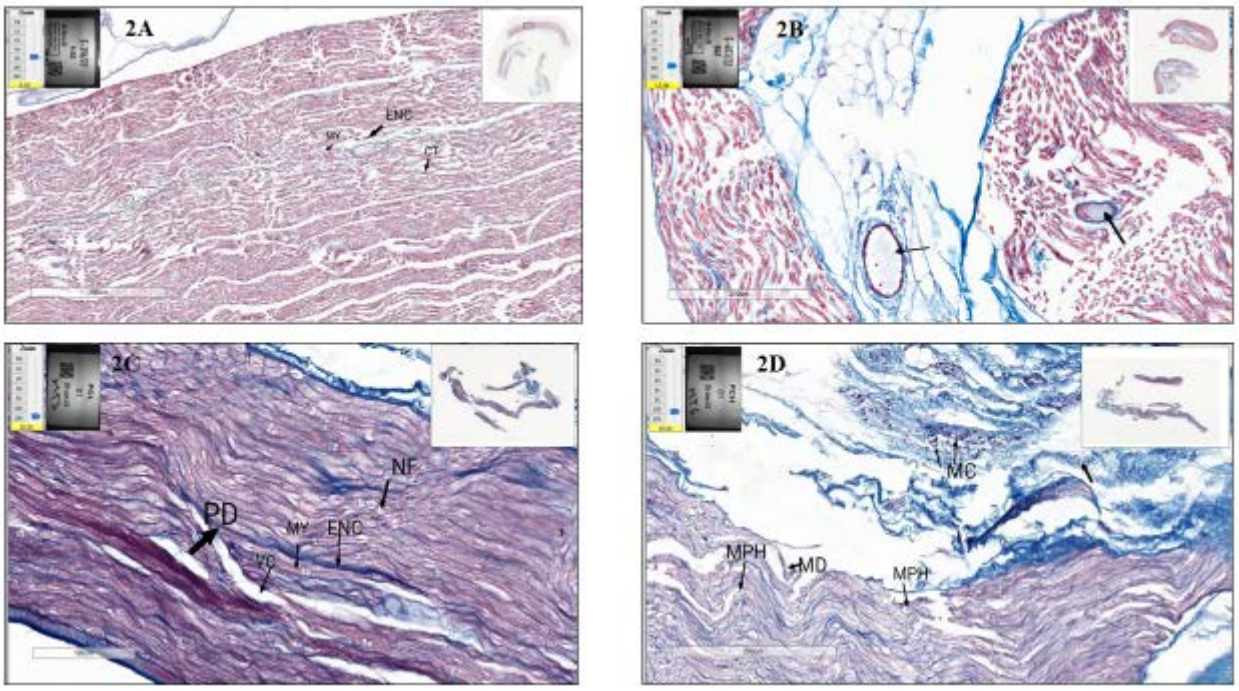


Figure 5. Light photomicrographs of the sciatic nerve were examined to assess the effect of different doses of *S. asoca* methanolic leaf extract- Masson's trichrome staining. 2A, Normal Control: demonstrating that the connective tissue, thin endoneurial collage, and myelin sheath enclosing the axonal appendages are all histologically and morphologically normal, exhibiting a decrease in the incidence of neurofibrillary tangles. 2B, Disease Control: showed increased levels of neurofibrillary tangles, which might be a sign of a neurodegenerative disease. Axons with pale colouring, different diameters, weak myelin sheaths, damaged nerve fibres, cytoskeletal filament accumulations, tubulovesicular profiles, granular/membranous entities, and malformed mitochondria are the characteristics of sciatic nerve neuroaxonal degeneration. 2C, 2D *S. asoca* (Both 200 and 400 mg/kg showed, that the myelin sheath degenerates, causing an increase in mass cells, collagen fibres, degenerative nerve bundles, and disruption of the myelin sheath, accompanied by specialized macrophages and a decrease in the prevalence of neurofibrillary tangles. X is represented by the scale bar at 200 µm, 300 µm, and 400 µm.

According to our findings, a 400 mg/kg dosage of *S. asoca* therapy experienced a reduced withdrawal response in the cold hyperalgesia nerve injury threshold at 4°C caused by cold pain and the tail experienced thermal hyperalgesia and immersion in a warm water bath. (46°C) showed reduced signs of struggle or withdrawal response in the tail. Some studies show that pain relief can activate brain pain inhibitory systems, leading to placebo-induced analgesia. Thermal hyperalgesia and tactile allodynia caused by peripheral nerve injury can be eliminated by microinjection of lidocaine or CCK2 antagonists into the RVM and physical disruption[49].

Through a complex pathomechanism including immunological responses, neuro-inflammation, DNA damage, myelin sheath damage, oxidative stress,

mitochondrial damage, and disruption of microtubules, chemotherapy drugs cause damage to the structures of the nervous system and cause chronic inflammatory peripheral neuropathy[50]. The best course of action would be to avoid CIPN by treating patients in advance with the right medications to lessen the severity or occurrence of CIPN. Chemotherapeutic medication cisplatin, produced from platinum, has antineoplastic effects, impaired general health, and toxic neuropathic pain. These side effects make over time neuropathy or analgesia therapy studies in rats more difficult[51].

This study demonstrates the neuroprotective potential of *Saraca asoca* methanolic leaf extract, attributed to its phytochemical profile, including flavonoids, alkaloids, tannins, and saponins. Flavonoids, especially quercetin dehydrate, play a key role in mitigating oxidative stress and neuroinflammation, which are central to neurodegenerative diseases. The anti-oxidative and anti-inflammatory properties of these compounds are likely to contribute to the observed neuroprotective effects[52].

Loss of motor coordination, unintentional weight loss, grip strength, running distance, and speed were found in the disease control group, whereas maximum walking speed on the Rotarod and measurement on the narrow beam walk was found to significantly increase with a dose of *Saraca asoca* therapy on 400 mg/kg body w.t.

Numerous scientific studies show that CRP is essential for the emergence of diseases and disorders[53]. Our research findings focus on systemic inflammation and evaluate how it affects pain and other symptoms from the perspective of CIPN.

Using Chromatography techniques like TLC, column chromatography and HPTLC assays, a methanolic extract of *S. asoca* verified the existence of quercetin dihydrate. The leaf's year-round availability makes the presence of quercetin dihydrate in it noteworthy. Therefore, additional quantification of the quantity of dihydrate quercetin dihydrate in *S. asoca* leaf extract is possible to ensure the correct use of this ancient plant.

In neural cells, the generation of ROS initiates a cascade that leads to apoptosis. It creates mediators of inflammation, which lead to the depletion of antioxidant enzymes, including superoxide dismutase, glutathione, alondialdehyde, and catalase. These antioxidant enzymes' disruption causes mitochondrial malfunction, demyelination of neurons, and extracellular matrix build-up[54]. On *S. asoca* leaf extract, the neuroprotective effects of reduced glutathione, elevated malondialdehyde, and enhanced catalase activity on CIPN were seen. This oxidative enzyme normalization offers neuroprotection by preventing demyelination.

Histological studies offer insightful data to support haematological and biochemical tests. Treatment with *S. asoca has* shown a robust shielding effect on the sciatic nerves. Hematoxylin and eosin stains during a histological study revealed intact myelin sheaths and retained nerve architecture. The *S. asoca* treatment significantly reduced inflammatory cell infiltration compared to the disease control group, which displayed degenerative changes like axonal swelling and myelin loss. The treated group's collagen distribution was more ordered according to Masson's trichrome staining, which may have a protective impact against fibrotic alterations. All things considered, our results suggest that *S. asoca* may help shield nerves from harm and neurodegeneration. The analysis of every damage to the sciatic nerve was provided through histopathology in Figures 4 and 5 and indicated that the internal organs of rats given *S. asoca* therapy had oedema, lesions, and anomalies. These results strongly imply that the extract could have neuroprotective qualities and might be harmful to the rat models' sciatic nerve[55].

Additional research is required to fully explore the therapeutic potential of *S. asoca* extract in treating neurodegenerative disorders. The physicochemical analysis in this study can assist in the identification of adulterants and inappropriate medication handling of plant material. In future research or applications, the current study can be a useful source of knowledge and standards for determining the quality of this plant material.

6. Conclusion

While the current findings highlight the neuroprotective potential of *S. asoca* methanolic leaf extract, mitigating cisplatin-induced peripheral neuropathy is promising. Future research should focus on isolating and characterizing the specific phytochemicals responsible for these effects. Numerous chemical components are found in the *S. asoca* and the presence of quercetin

dihydrate as the main flavonoid, is likely responsible for its pharmacological effects. Current research indicates that the extract reduces oxidative stress and stimulates nerve regeneration, which helps to lessen the structural and functional harm that CIPN causes. Comparative studies with established neuroprotective agents will also be essential to validate its efficacy and expand its therapeutic potential in treating neurodegenerative conditions and chemotherapy-induced neuropathy.

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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.

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