

Casticin and Cynarin Attenuate Cisplatin-Induced Peripheral Neuropathy in Wistar Rats via Modulating Neuroinflammatory Pathways

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Running Title: Casticin and Cynarin Protect Against Cisplatin-Induced Neuropathy

ABSTRACT

Background: Chemotherapy-induced peripheral neuropathy [CIPN] is neurological disorder related to cancerous treatment with drugs like cisplatin. Cisplatin-induced neurotoxicity is mainly associated with oxidative stress, inflammatory responses, and structural alterations in peripheral nerves. Natural bioactive compounds such as casticin, a flavonoid, and cynarin, a quinic acid derivative, have demonstrated significant neuroprotective potential as well as antioxidant and anti-inflammatory potential during preclinical studies.

Methodology: Peripheral neuropathy was introduced in animals by intraperitoneal injection of cisplatin [2 mg/kg twice weekly] for consecutive 28 days. Experimental rats were injected with pregabalin [30 mg/kg, p.o.] as standard treatment, casticin [7.5, 15, and 30 mg/kg, p.o.], cynarin [5, 10, and 20 mg/kg, i.p.]. Behavioural parameters including mechanical allodynia, thermal hyperalgesia, motor coordination and nerve conduction velocity and locomotor activity were evaluated. Furthermore, various markers of neuroinflammations like TNF- α and IL-6 were studied followed by histopathology of sciatic nerve tissue, were assessed.

Results and Discussion: Cisplatin administration produced significant neuropathic alterations characterized by reduced pain threshold, impaired motor coordination, decreased locomotor activity, reduced motor nerve conduction velocity, elevated inflammatory cytokine levels, and sciatic nerve degeneration. Treatment with casticin and cynarin significantly attenuated cisplatin-induced neuropathic changes by improving behavioral responses, reducing TNFs- α and IL-6 levels, and preserving sciatic nerves architecture. These protective effects may be associated with suppression of neuroinflammatory pathways and restoration of peripheral nerve integrity.

Conclusion: The present findings suggest that casticin and cynarin exhibit significant protective effects against cisplatin-induced peripheral neuropathy. These phytoconstituents can serve as a potential pharmacological agent for the management of CIPN through modulation of inflammatory mechanisms.

Keywords: Chemotherapy-induced peripheral neuropathy, Hyperalgesia, allodynia, Cisplatin, Casticin, Cynarin, Neuroinflammation, Sciatic nerve

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INTRODUCTION

Neuronal or Neuropathic pain is a complex neurological disorder which may be the result of injury and dysfunction of the neurons and is commonly associated with altered sensory responses, including hyperalgesias allodynia's. It has been observed that nearly 7-8% general population's life is get affected and significantly reduces quality of life [1]. Cancer is considered as a worldwide health problem which is the major reason for causing the deaths. In 2020, it has been observed that nearly 19.3 million population was diagnosed as a cancer. And out of these nearly 10 million peoples were died due to cancer disease worldwide. Despite the growing incidence of cancer, improvements in diagnostic techniques and therapeutic strategies have contributed to better survival outcomes.[2] Cancer is still considered as major health challenges worldwide, and chemotherapeutic agents are extensively used for the treatment of various malignancies. However, several anticancer drugs produce dose-limiting neurotoxicity resulting in sensory abnormalities, motor dysfunction, and peripheral nerve damage [3]. Chemotherapeutic agents cause frequent adverse effects leading to impaired motor and sensory function and peripheral neuropathy; the exact pathophysiology is not known [4]. Cisplatin is most widely used anticancer agent for the treatment of malignant tumors, including testicular, ovarian, bladder, and lung cancer [5]. Cisplatin mediated PN is related to the oxidative stress, mitochondrial dysfunction, neuroinflammation, neuronal damage, leading to impaired nerve function and neuropathic pain symptoms. Symptoms of CIPN usually appear during chemotherapy and within a few weeks after treatment ends. Within the first month after chemotherapy, 68% of patients may experience CIPN. Although CIPN symptoms may gradually decrease within 3–6 months after completion of chemotherapy in some patients, persistent symptoms can remain depending on treatment duration, cumulative dose, and individual patient characteristics.[6] However, depending on treatment duration and dose, patient characteristics, symptoms may last for months or even become permanent; these symptoms can reduce a patient's quality of life and interfere with daily social work activities. Cancer patients with CIPN have approximately 2.5 times greater risk of falling than those without CIPN. Tools for measuring and assessing CIPN are important for both clinical practice and research purposes [4]. CIPN is a kind of toxicity related to the various anticancer agents like platinum compounds, bortezomib, thalidomide taxanes, and vinca alkaloids. The neurotoxic effects of these therapeutic agents always require reduction in dose or discontinuation in treatment which ultimately leads to compromise the outcome and substantially diminishing patients' quality of life.[7]. Hence, identifying effective therapeutic strategies targeting

chemotherapy-induced peripheral neuropathy remains an important area of research. Casticin, also called vitexicarpin, is [3',5'- dihydroxy-3,4',6,7-tetramethoxyflavone]. It is a naturally occurring flavonoid mainly isolated from *Fructus viticis* and has gained attention due to its diverse pharmacological activities. Casticin has antioxidant, anti-inflammatory, anticancer as well as cytoprotective properties, which may contribute to its potential neuroprotective activity. [8]. Cynarin, known as *Cynara scolymus* as an ancient herb that originated from the southern Mediterranean part of North Africa. Cynarin possesses several pharmacological properties, including hepatoprotective activities, suggesting its excellent contribution in reducing oxidative and inflammatory damage [9]. Increasing evidence indicates that neuroinflammation plays a central role in progression of CIPN. Among inflammatory mediators, TNF- α acts an early pro-inflammatory cytokine that activates intracellular signalling pathways such as NF- κ B, results in enhanced cytokine expression including IL-6. Elevated TNF- α and IL-6 enhance oxidative stress, activate glial and immune cells, sensitize nociceptive neurons, and promote axonal degeneration, thereby contributing to neuropathic pain and impaired peripheral nerve function. Therefore, suppression of TNF- α and IL-6 signalling represents an important therapeutic strategy for preventing cisplatin-induced neuroinflammation and peripheral nerve injury [10–13].

MATERIAL AND METHODS

Drug and Chemical

Cisplatin [Neon laboratories Ltd., Mumbai], Pregabalin [Sun Pharma], Casticin [National Analytical Chemicals], Cynarin [Phytolab]. All drugs and other materials utilised in present research work of AR grade and sourced from standard commercial suppliers.

Animals

For the study, Albino Wistar rats [220- 250 g] were used. A total of 54 animals were utilised in research work which were arranged in 9 groups each containing 6 animals in propylene cages. The experiments were carried out under standard laboratory environmental conditions [25 ± 2 °C/ $55 \pm 5\%$ RH]. During experiments all rats were fed with laboratory diet as well as water. The study protocol was authorised by the research centre MVP's College of Pharmacy, Nashik-422002 with protocol approval of IAEC/Oct2024/14. All animal experiments were performed as per guidelines issues by CCSEA, New Delhi.

Experimental Design

The disease was induced by injecting Cisplatin [2mg/kg twice a week for 28 consecutive days] in experimental animals.[14]. During experiments weight loss, balance deficits, as well as decreased

locomotion were evaluated. The phytoconstituents casticin [7.5, 15, and 30 mg/kg, p.o.] and cynarin [5, 10, and 20 mg/kg, i.p.] was injected concomitantly with cisplatin treatment for 28 days. Pregabalin [30 mg/kg, p.o.] was considered as standard during experiments. Behavioural parameters like Mechanical allodynia, hyperalgesia, motor nerve conduction test, rota rod, and actophotometer were used to evaluate the impact of test drug on chemotherapy-induced peripheral neuropathy.

Group No.	Groups detail	Dosing details
Group I	Normal Control	D/W [p.o.]
Group II	Disease Control	Cisplatin [2mg/kg, i.p. twice a week] for 4 consecutive weeks.
Group III	Casticin [low dose]	Cisplatin [2mg/kg, i.p. twice a week] + CST [7.5 mg/kg p.o.] for 4 consecutive weeks.
Group IV	Casticin [medium dose]	Cisplatin [2mg/kg, i.p. twice a week] + CST [15 mg/kg p.o.] for 4 consecutive weeks.
Group V	Casticin [high dose]	Cisplatin [2mg/kg, i.p. twice a week] + CST [30 mg/kg p.o.] for 4 consecutive weeks.
Group VI	Cynarin [low dose]	Cisplatin [2mg/kg, i.p. twice a week] + CYN [5 mg/kg i.p.] for 4 consecutive weeks.
Group VII	Cynarin [medium dose]	Cisplatin [2mg/kg, i.p. twice a week] + CYN [10 mg/kg i.p.] for 4 consecutive weeks.
Group VIII	Cynarin [high dose]	Cisplatin [2mg/kg, i.p. twice a week] + CYN [20 mg/kg i.p.] for 4 consecutive weeks.
Group IX	Standard	Cisplatin [2 mg/kg, i.p. twice weekly] + Pregabalin [30 mg/kg, p.o.] for 4 consecutive weeks.

The behavioural properties were screened at end of 1st to 4th weeks of treating. After 4 weeks, rats were euthanized using a CO₂ euthanasia chamber on the last day, 1 h after all behavioral tests, sciatic nerves were separated cautiously to prepare homogenate of tissue in 0.1 M

Tris-HCl buffer [pH 7.4] and finally, molecular and biochemical estimations were performed.

Methods

Body weight determination-Prior to the initiation of treatment, all animals were weighed, and those with similar body weights were distributed among the experimental groups. Animals were randomized before treatment administration, and body weight measurements were obtained periodically throughout the study until its conclusion [15,16].

Rota rod: This was used to determine motor coordination's according to the previously reported method. Animals were placed for one minute on a revolving rod 30 or 60 minutes after IP or oral administration of the drug [17]. and recorded how many animals fell off the roller. The rod was rotated at 4 rpm at first, then at 20 rpm. The latency to fall from the continuously accelerating rotating rod was recorded for each animal [18]

Hot Plate: Nociceptive response was evaluated using Eddy's hot plate maintained at 55 ± 1°C. Animals were individually placed on the heated surface, and the latency to the initial paw licking or jumping response was recorded. A 10-s cut-off was established to minimize the risk of paw injury [19].

Acetone drop test [Cold allodynia]: -

Cold sensitivity was evaluated by gently applying 50 µL of acetone to the mid-plantar surface of the hind paws. The evaporative cooling effect developed within 2–5 s, eliciting nociceptive behaviors such as paw withdrawal, shaking, rubbing, or licking. Responses from both paws were recorded every 5 min, and the mean value was used for subsequent analysis [17].

Von Frey test [Mechanical allodynia]:

In this test animals were kept in separate plexiglass boxes mounted on mesh floor of stainless-steel and adjusted for 20 min. Mechanical nociceptive threshold was determined using calibrated von Frey monofilaments. Each filament was applied perpendicular to the plantar surface of the hind paw with enough pressure to bend the filament for 6 s. Paw withdrawal indicated a positive response. If withdrawal occurred, testing continued with the next lower-force filament; otherwise, the next higher-force filament was used. Measurements were repeated four to five times at 5-min intervals, and the mean withdrawal threshold was calculated [20,21].

Actophotometer [Locomotor activity]:

The actophotometer was utilised to estimate the activity which contained photoelectric cells connected to circuit along with counter. Animals were observed for 5 min, and each break in the photocell light beam caused by their movement was automatically recorded as an activity count [22].

Motor nerve conduction velocity [MNCV]

On 28th day of Disease induction, Animals were

anesthetized using ketamine [90 mg/kg, i.p.] and xylazine [10 mg/kg, i.p.] before recording MNCV. The MNCV was determined using an 8-channel Power Lab AD system. This instrument was equipped with specialized electrode [MLA0320] that can stimulate the animal nerves. Apart from this electrode needle [MLA1204] was also associated to record the readings. The proximal end of the nerve was stimulated using a stimulating electrode, whereas the electrical response was recorded from a recording electrode positioned at the distal end. Motor nerve conduction velocity was calculated by dividing the inter-electrode distance [m] by the latency period [s] [23,24].

Estimation of TNF- α and IL-6 levels

ELISA kit was used to quantify the TNF- α and IL-6 present in tissue homogenate of sciatic nerve. The experiments were performed as per instructions provided by manufacturer. [13]

Histopathology:

Animals used in experiments were sacrificed carefully, and the sciatic nerves separated and rapidly fixed in 10% formalin. Fixed sciatic nerve tissues were processed through dehydration, paraffin embedding, sectioning, and staining procedures for microscopic evaluation. Section fixed in paraffin blocks. Stained with haematoxylin and eosin and observed. Photomicrographs of sections were used for evaluation. A digital microscope [Olympus, Japan] was used to analyze the sections under a light microscope while taking photomicrographs [25].

Statistical Analysis

Results are reported as mean $\pm$ SEM. Statistical computations were performed using GraphPad Prism version 11. Comparisons between groups were analyzed using one-way ANOVA, followed by Dunnett's multiple comparison test. Statistical significance was assigned at $p < 0.05$, with increasing levels of significance indicated by $p < 0.01$, $p < 0.001$, and $p < 0.0001$ when compared with the disease control group.

RESULTS

Impact of casticin and cynarin on body weight

Cisplatin administration produced significant decrease in body weight [#### $p < 0.0001$] as compared to normal control group [NCG]. Animals treated with casticin, cynarin, and pregabalin significantly improved body wt in comparison to disease control group [DCG]. Casticin [7.5, 15, 30 mg/kg], Cynarin [5, 10, 20 mg/kg], and Standard treatment demonstrated significant enhancement in body weight [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] in comparison to disease control group. [Figure A]

Impact of casticin and cynarin on fall-off latency in rota rod test

Disease control rats showed motor incoordination as indicated by a significant [p<0.0001####] decline fall

latency [sec] in comparison to the NCG. Animals treated with std and test group showed significant [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] enhancement in coordination motor, demonstrated as enhanced fall latency time[sec] after the 3rd week of the treatment schedule in comparison to the DCG. [Figure B]

Impact of casticin and cynarin on paw withdrawal latency in hot plate test

Disease Control rats showed significant reduction [p<0.0001####] in paw withdrawal latency from 2nd week of Cisplatin injection in comparison to NCG. Animals treated with pregabalin, casticin [7.5, 15, and 30 mg/kg], and cynarin [5, 10, and 20 mg/kg] significantly increased paw withdrawal latency [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] in comparison to DCG [Figure C]

Impact of casticin and cynarin on allodynia score in acetone drop test

Rats in the disease control group exhibited significant cold allodynia by the **second week** following disease induction. A reduction in paw withdrawal latency [s] was considered indicative of cold allodynia. Animals from DCG showed significant reduction [p<0.0001####] in paw withdrawal latency at the 2nd week of Cisplatin injection in comparison to NCG. Treatment with std and test group showed significant [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] improvement in paw withdrawal latency at the 3rd and 4th weeks of treatment schedule as compared to DCG [Figure D]

Impact of casticin and cynarin on locomotor counts in actophotometer test

Effect of casticin and Cynarin on locomotor activity. Disease control rats show significant [p<0.0001####] lowering locomotor count in comparison to NCG. Animals treated with std and test group showed significant [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] improvement in locomotor counts in comparison to DCG after 3rd week of treatment schedule. [Figure E]

Impact of casticin and cynarin on Mechanical allodynia

It has been observed that, at the end of four weeks, Cisplatin injection demonstrated significant lowering of [p<0.0001####] mean paw withdrawal threshold of cisplatin group in comparison to vehicle-treated group. Cisplatin-injected rats, treated with test group, demonstrated significant dose-dependent increase in mean paw withdrawal threshold [* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$] in comparison to DCG. Also, treatment with the standard [Pregabalin 30 mg/kg] group for 4 wks enhanced mean paw withdrawal threshold [**** $p < 0.0001$] in comparison to DCG [Figure F]

Impact of casticin and cynarin on MNCV

Casticin and Cynarin Attenuate Cisplatin-Induced Peripheral Neuropathy in Wistar Rats via Modulating Neuroinflammatory Pathways

Disease control animals showed significant [$p < 0.0001^{####}$] reduction in NCV after 28th day of Cisplatin treatment, which indicates lesser damage to nerves in comparison to NCG. Cisplatin-injected animals, treated with std and test groups, showed significant [$*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$] improvement in NCV in comparison to DCG [Figure G].

HISTOPATHOLOGICAL ANALYSIS

The microscopic observations of sciatic nerve samples of the NCG indicated normal neuronal fibers, axons, nuclei. Sciatic nerve from the DCG showed necrosis, neuronal degeneration, vacuolation, and inflammation when compared with Normal Control group animals. Compared with the DCG, treatment with Casticin [7.5, 15, and 30 mg/kg, p.o.] and Cynarin [5, 10, and 20 mg/kg, i.p.] produced a dose-dependent reduction in both the incidence and severity of the observed lesions, indicating that these pathological changes were reversible. Based on these histopathological observations, it can be determined that animals injected with the test compound, specifically casticin [30 mg/kg, p.o.] and cynarin [20 mg/kg, i.p.], exhibited an ameliorative effect and demonstrated significant protective effects on sciatic nerve tissue.

Figure 1: Effect of casticin and cynarin treatment on cisplatin-induced behavioural and functional alterations in Wistar rats.

[A] Body weight changes

[B] Rota rod fall-off latency

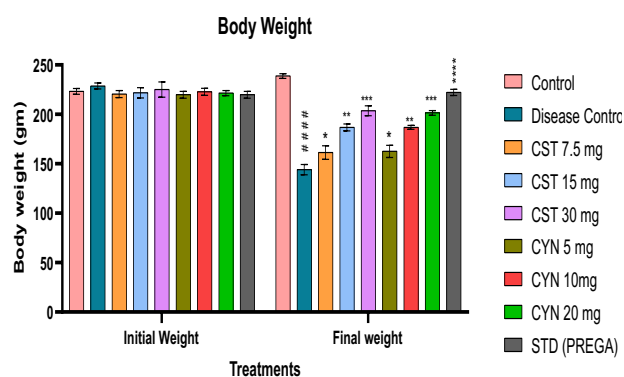
[C] Paw withdrawal latency in hot plate test

[D] Cold allodynia response

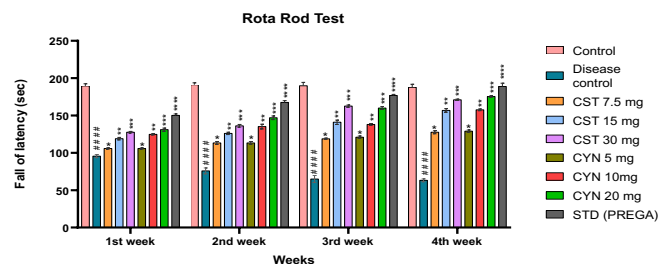
[E] Locomotor activity using actophotometer

[F] Mechanical allodynia using Von Frey filament test

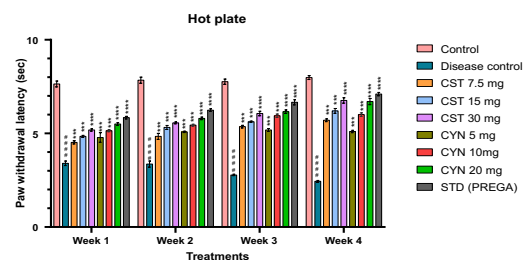
[G] MNCV



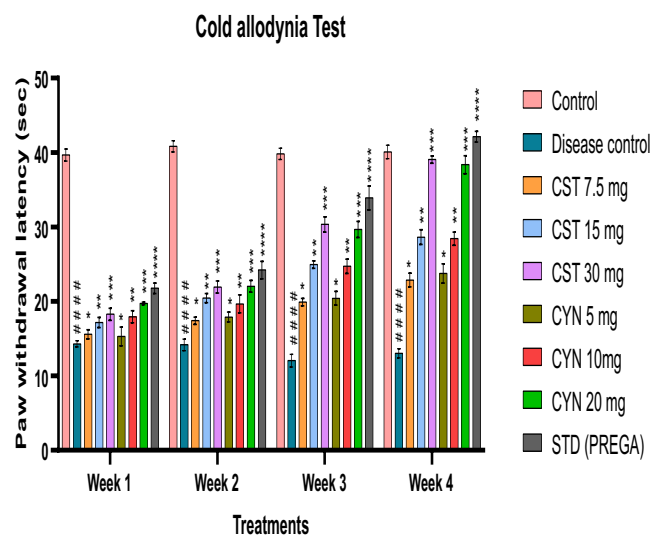
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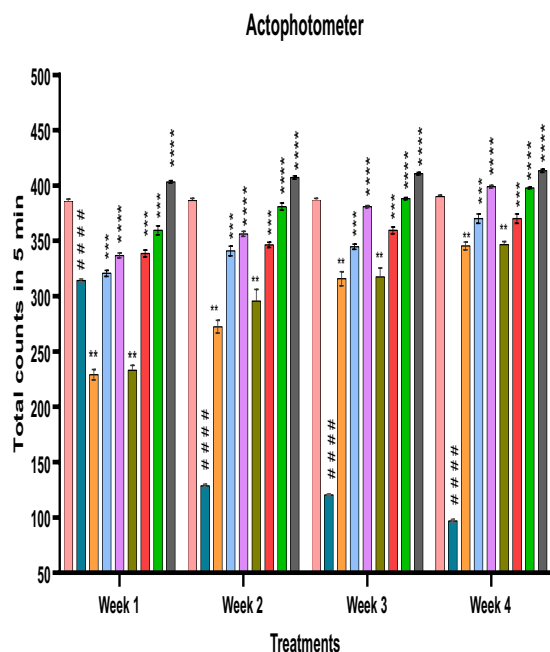
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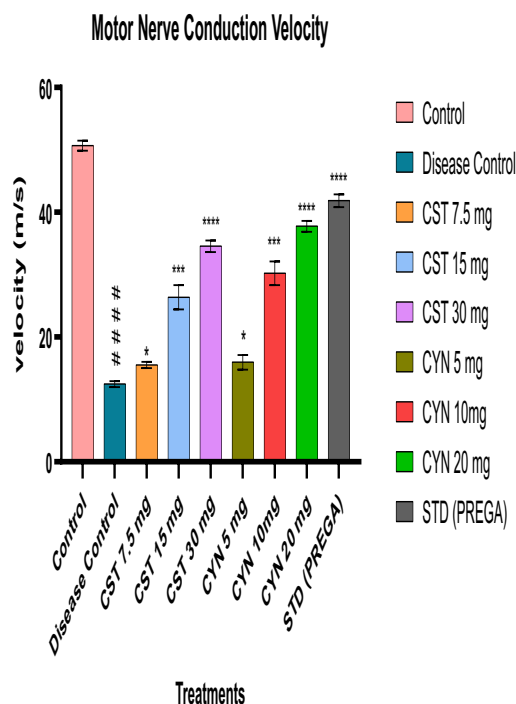
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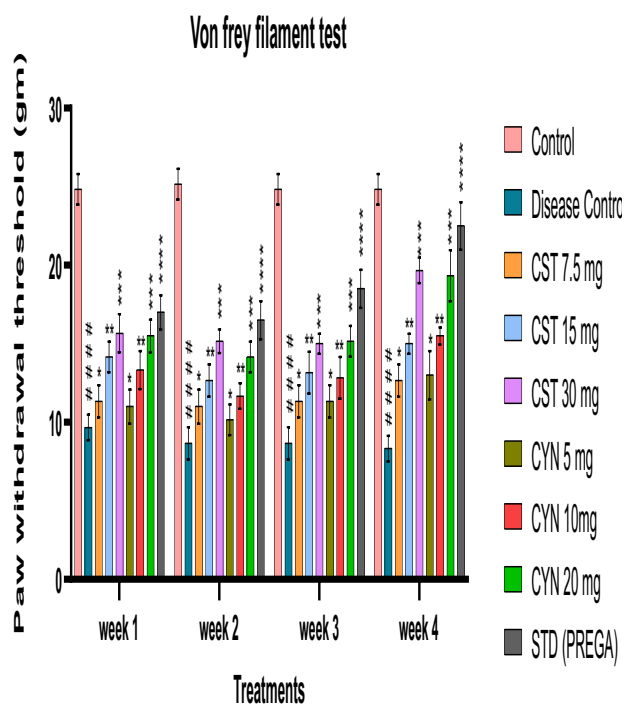
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[E]

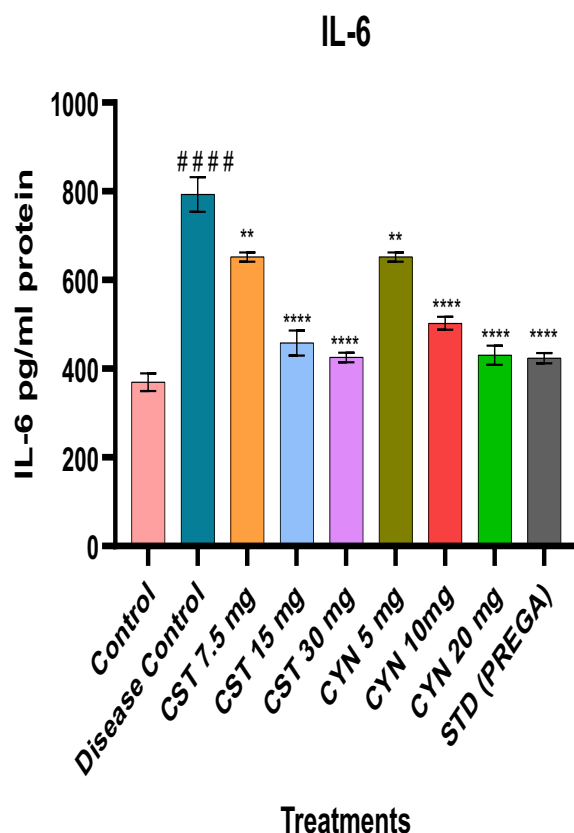
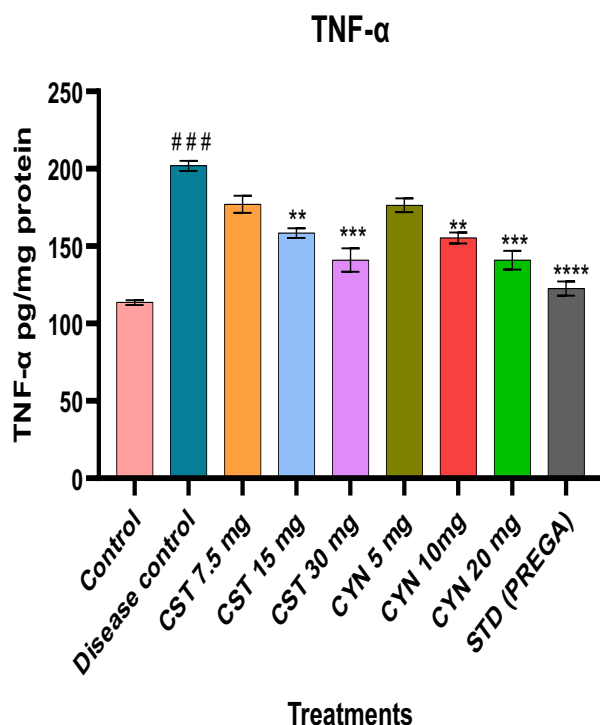


[G]



[F]

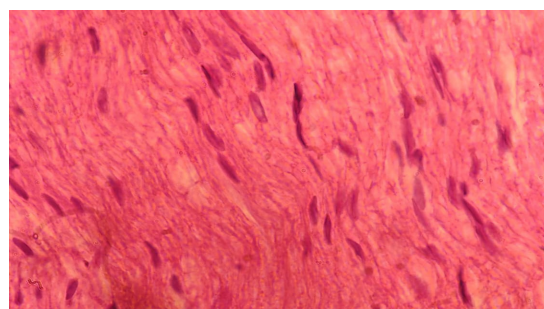
Figure 2: Impact of casticin, cynarin treatment on inflammatory biomarkers TNF- α and IL-6 levels in cisplatin-associated peripheral neuropathy. All observations presented as mean $\pm$ SEM [n=6]. #####p<0.0001 in comparison to NCG; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with DCG.

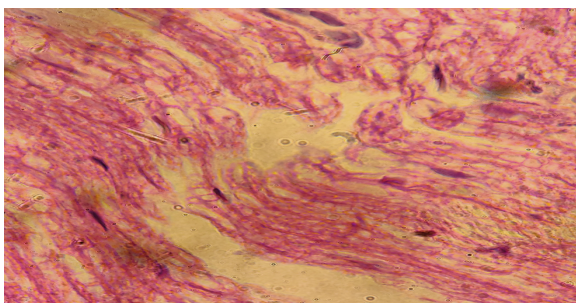


Cisplatin-injected rats had significantly [$P < 0.0001$ ####] increased IL-6 and TNF- α in comparison to normal controlled rats. However, treating with standard as well as test group indicated significant [$*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$] decline in IL-6 and TNF- α in comparison to DCG. Oxidative stress induced by cisplatin activates inflammatory signalling pathways and causes generation of TNF- α and IL-6. TNF- α serves as an upstream inflammatory mediator capable of activating NF- κ B signalling, which subsequently enhances IL-6 expression and amplifies inflammatory cascade. Persistent elevation of such cytokines contributes to neuronal inflammation, mitochondrial dysfunction, Schwann cell injury, and axonal degeneration, ultimately showing in neuropathic pain. The significant reduction of TNF- α and IL-6 following treatment with casticin and cynarin suggests effective attenuation of cisplatin-induced neuroinflammation. It is also suggested that inflammation can directly contribute to cisplatin-induced neuropathy. TNF- α contributes to cisplatin-induced neuropathy by promoting inflammation, oxidative stress, and neuronal damage, leading to impaired nerve function and enhanced neuropathic pain.

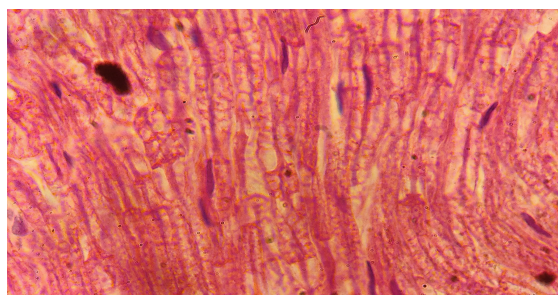
Figure 3: Histopathological examination of sciatic nerve sections stained with hematoxylin and eosin [H&E].

[I] Normal control
 [II] Cisplatin-induced disease control
 [III] Treatment with Casticin 7.5 mg/kg
 [IV] Treatment with Casticin 15 mg/kg
 [V] Treatment with Casticin 30 mg/kg
 [VI] Treatment with Cynarin 5 mg/kg
 [VII] Treatment with Cynarin 10 mg/kg
 [VIII] Treatment with Cynarin 20 mg/kg
 [IX] Treatment with Pregabalin 30 mg/kg
 Arrows indicate changes in nerve fiber arrangement, axonal integrity, and histological architecture.

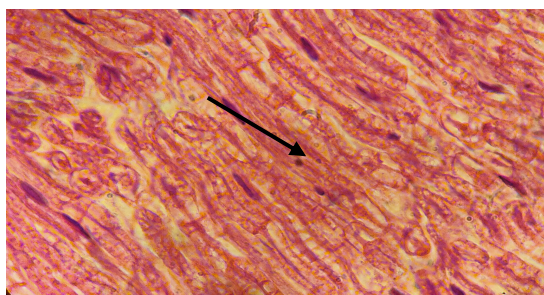




I

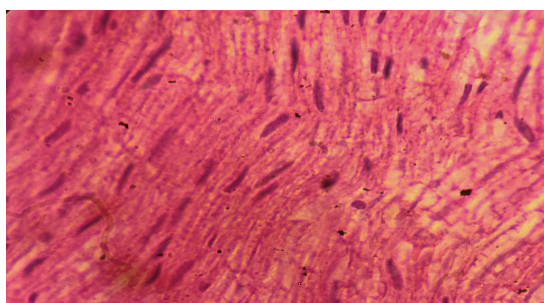


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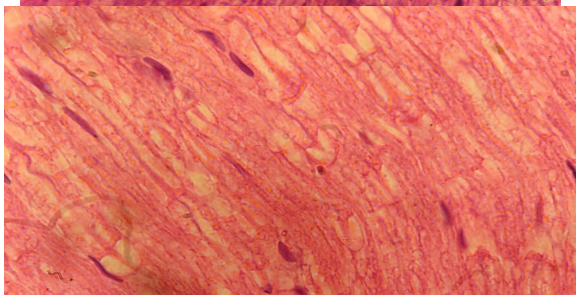


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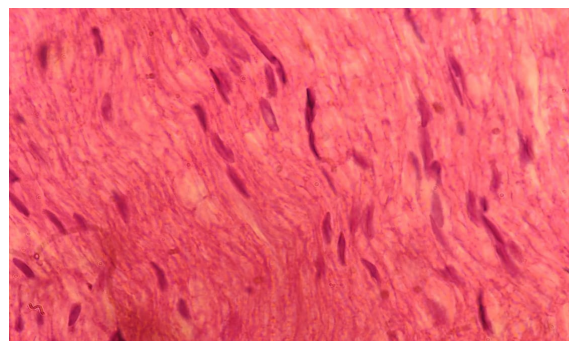
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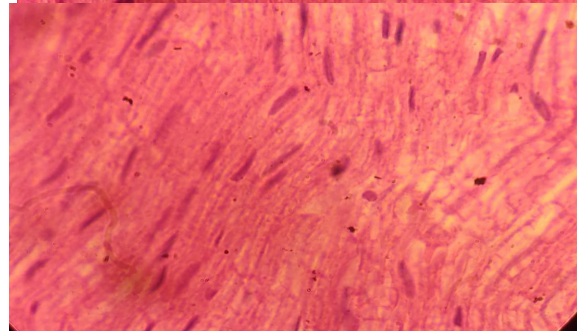
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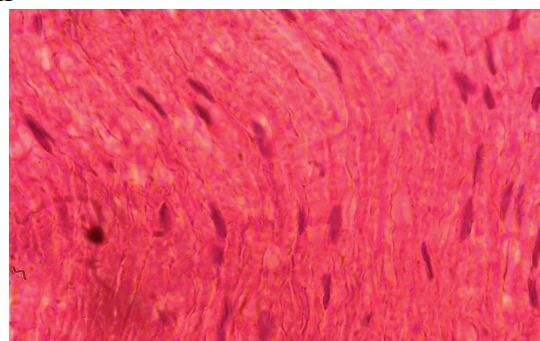
VI



VII



VIII



IX

I: Normal control – Sciatic Nerve showing normal histology, well-organized myelin sheath, axons nerve architecture, with no signs of mononuclear cell infiltration, axonal irregularities, or degeneration. The myelin sheath, axons, and Schwann cell nuclei appeared histologically intact and unaltered.

II: Cisplatin group - sciatic nerve showing highly separate nerve fibres, axonal irregularity, and degeneration and mononuclear cellular infiltrations; cisplatin-treated rats showed the presence of mononuclear cellular infiltration, axonal degeneration, and widely separated nerve fibres after 4 weeks of the study.

III: Cisplatin + CST [7.5 mg/kg] - Sciatic Nerve showing highly separate nerve fibres, axonal irregularity, and degeneration; CST [7.5 mg/kg] administration for 4 weeks showed mild axonal regeneration, with a high separation of nerve fibres and mononuclear cellular infiltrations.

IV: Cisplatin + CST [15 mg/kg] - Sciatic Nerve showing well-organized myelin sheath, slightly separated nerve fibers; treatment with CST [15 mg/kg] showed moderate abrogation of the disturbed histopathological picture with slightly separated nerve fibers.

V: Cisplatin + CST [30 mg/kg] - Sciatic Nerve showing well-organized myelin sheath and Schwann cell nuclei; Treatment with casticin at a dose of 30 mg/kg produced the most favorable histological profile, evidenced by significant enhancement of axonal regeneration, preservation of nerve fiber continuity, and absence of mononuclear cell infiltration.

VI: Cisplatin + CYN [5 mg/kg] - Sciatic Nerve showing highly separated nerve fibres, axonal irregularity, and degeneration. CYN [5 mg/kg] administration for 4 weeks showed mild axonal regeneration, with a wide separation of nerve fibres and mononuclear cellular infiltrations.

VII: Cisplatin + CYN [10 mg/kg] - Sciatic Nerve showing a well-organized myelin sheath, slightly separated nerve fibres. Treatment with CYN [10 mg/kg] showed moderate abrogation of the disturbed histopathological picture with slightly separated nerve fibres.

VIII: Cisplatin + CYN [20 mg/kg] - Sciatic Nerve showing a well-organized myelin sheath and Schwann cell nuclei; Treatment with cynarin at a dose of 20 mg/kg produced the most favorable histological profile, evidenced by significant enhancement of axonal regeneration, preservation of nerve fiber continuity, and absence of mononuclear cell infiltration.

IX: Cisplatin + Pregabalin [30 mg/kg] - Sciatic Nerve showing well-organized myelin sheath and Schwann cell nuclei; Pregabalin-treated animals showed well-organized myelin sheath, axons, and Schwann cells without any separation of nerve fibers.

DISCUSSION

The findings of the present investigation indicate that casticin and cynarin administration reduced cisplatin-associated neuropathic alterations in experimental animals. Cisplatin administration produced characteristic neuropathic alterations including reduced pain threshold, impaired motor coordination, decreased locomotor activity, reduction in MNCV, and structural damage in tissues of sciatic nerves. Development of cisplatin-associated peripheral neuropathy is mainly related to oxidation related stress, inflammatory pathway activation, as well as neuronal injury. Enhancement in levels of TNF- α and IL-6 plays role in nerve damage by promoting neuroinflammatory responses and impairment of

normal nerve function. TNF- α and IL-6 are closely interconnected mediator of inflammation during the progression of chemotherapy-induced peripheral neuropathy. TNF- α stimulates activation of NF- κ B and MAPK signalling pathways, leading to increased synthesis of IL-6. Elevated IL-6 further enhances inflammatory cell recruitment, neuronal sensitization, and oxidative stress, creating a positive feedback loop that sustains peripheral nerve injury. Therefore, simultaneous suppression of both TNF- α and IL-6 observed in current research work indicates effective modulation of neuroinflammatory pathways, which may explain the improvement in behavioural parameters, preservation of MNCV, and restoration of sciatic nerve histology.

Treatment with casticin and cynarin significantly improved behavioural parameters and MNCV in comparison to DC animals. The observed improvement might be due to the antioxidant as well as anti-inflammatory properties, resulting in reduced inflammatory cytokine expression and protection of sciatic nerve architecture.

Histopathological observations further supported the protective effects of casticin and cynarin by showing improvement in axonal integrity, preservation of myelin structure, and reduction of inflammatory changes compared with cisplatin-treated animals.

Previous investigations have demonstrated that inhibition of TNF- α -mediated signalling reduces inflammatory cytokine release, oxidative stress, and neuronal apoptosis in experimental neuropathy models. Cynarin inhibits microglia-induced pyroptosis and neuroinflammation via Nrf2/ROS/NLRP3 axis after spinal cord injury [26]. Likewise, suppression of IL-6 has been associated with reduced peripheral nerve inflammation and improved functional recovery. The observed reduction in both TNF- α and IL-6 following casticin and cynarin treatment is therefore consistent with their reported anti-inflammatory and antioxidant activities and additionally supports their capability as neuroprotective molecule against cisplatin-induced peripheral neuropathy.

In conclusion, casticin and cynarin may provide neuroprotection against chemotherapy-induced peripheral neuropathy through modulation of inflammatory pathways. Further studies involving detailed molecular mechanisms may establish their pharmacological potential.

CONCLUSION

The outcomes of this study indicate that casticin and cynarin exert protective effects against cisplatin-induced peripheral neuropathic changes

in Wistar rats. Treatment improved behavioural abnormalities, restored motor nerve conduction velocity, reduced TNF- α and IL-6, and protected sciatic nerve architecture. The neuroprotective effects may be associated with suppression of neuroinflammation and preservation of neuronal integrity. Overall, the results indicate that casticin and cynarin may serve as effective candidates for alleviating chemotherapy-induced peripheral neuropathy.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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Author Contributions

Author Name performed the experimental work, collected data, performed analysis, interpreted the results, and contributed to manuscript preparation.

Author Name contributed to study design, supervision, interpretation of experimental findings, and critical revision of the manuscript.

All authors have read and approved the final version of the manuscript.

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