Preclinical assessment of Casticin and Cynarin for Neuroprotection in Streptozotocin-Induced Model of Diabetic Neuropathy

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ABSTRACT

Diabetic neuropathy (DN) is a common debilitating complication of diabetes mellitus, characterized by progressive nerve damage resulting from chronic hyperglycaemia-induced oxidative stress, inflammation, and macrovascular dysfunction. Casticin, a flavonoid and cynarin, a caffeoylquinic acid derivative have demonstrated significant antioxidant, anti-inflammatory and neuroprotective effect in preclinical studies. So, this study was to evaluate the effect of Casticin and Cynarin on STZ induced diabetic neuropathy assessed by behavioural, biochemical , histopathological and electrophysiological changes. In Wistar rats diabetes was induced by using single dose of STZ (55mg/kg i.p). After induction of diabetes, animals are treated with Pregabalin (30mg/kg p.o), Casticin (7.5, 15, 30 mg/kg p.o) and Cynarin (5, 10, 20 mg/kg i.p) for next 4 weeks. This study explores the potential of casticin and cynrain as therapeutic agent in the management of diabetic neuropathy. Their mechanism of action appears to involve attenuation of oxidative stress, inhibition of pro-inflammatory cytokines (e.g, TNF-α, IL-6), Preliminary evidence from In-Vitro and In-Vivo models suggest that these phytochemicals improve nerve conduction velocity, reduce thermal hyperalgesia and preserve peripheral nerve architecture in diabetic condition.

Keywords- Diabetes, Hyperglycaemia, Hperalgesia, neuropathy, Casticin

INTRODUCTION

Diabetes has transformed into a metabolic epidemic projected to affect 366 million individuals by 2050. Diabetic retinopathy, neuropathy, nephropathy and cardiomyopathy are the most frequent pathological features of diabetic complications appearing in around 50% diabetics [1]. Diabetic neuropathy (DN) could define as a neuropathic disorder that affects all types of peripheral nerves, including sensory, motor and autonomic nerves, thus affecting nearly all body organs and systems. Moreover, DN usually affects the long somatosensory nerves of hand and feet leading to either loss of sensation or severe pain [2]. DN pains clinically presented with a lot of symptoms and signs such as burning, numbness, tingling, stabbing sensation, shooting pain, allodynia and hyperalgesia, with the failure of the endogenous analgesic pathways for controlling the neuropathic pain [3]Prolonged hyperglycemia disrupts the metabolic and/or mitochondrial redox state of the cell and leads to excessive for mation of reactive oxygen species (ROS) [4]. This increased pro duction of ROS triggers the activation of five major pathways involved in the pathogenesis of complications: polyol pathway flux, increased formation of advanced glycation end-products (AGEs), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C (PKC) iso forms, and overactivity of the hexosamine pathway [5]. ROS also activate a transcription factor NF-κβ which increases the production of TNF-α, IL-1β [6].

At present, only seven agents have been approved for the treatment of neuropathic pain in the U.S. These are lidocaine (5%) administered via a transdermal patch, gabapentin, duloxetine, pregabalin, gabapentin administered in a gastroretentive form, capsaicin (8%) administered via a transdermal patch, and tapentadol. It is notable that of these seven agents, only three, pregabalin, duloxetine, and tapentadol, have regulatory approval in the U.S. for the treatment of painful diabetic peripheral neuropathy [7].In the clinical setting, despite the use of these agents, the successful therapy of diabetic neuropathy remains a challenge. Due to pathogenic complexity of diabetic neuropathy, new therapeutic interventions targeting primary mechanisms contributing to nerve damage are critical for the future treatment of this complication [8]. Casticin is a polymethylflavone, a type of flavonoid, found in various plants, particularly those in the Vitex genus (*Artemisia annua*). It has been studied for its potential anticancer and anti-inflammatory properties, as well as other pharmacological effects

like anti-hyperprolactinemia [9]. Casticin is known for its ability to induce apoptosis (programmed cell death) in cancer cells and inhibit their growth [10]. Cynarin is a derivative of hydroxycinnamic acid and it has biologically active functional groups constituent of some plants and food. It's known for its antioxidant, anticholinergic, and anti-inflammatory properties. It also demonstrates potential hepatoprotective, hypocholesterolemic, and even anti-HIV effects [11]. The present study was designed with an objective to investigate the effects of casticin and cynarin in STZ-induced diabetic neuropathic pain in rats.

MATERIALS AND METHODS

Drugs and Chemicals: Casticin, Cynarin (National Analytical Corporation, Mumbai); Streptozotocin (Otto chemie Pvt Ltd, Mumbai); Nicotinamide (Kanha Biogenetic, Solan); Pregabalin (Sun pharmaceuticals, Gujrat); Nitrobluetetrazolium Chloride (NBT) (Himedia Laboratories Pvt. Ltd. Mumbai, India); thiobarbituric acid (TBA) (Research-Lab Fine Chem Industries, Mumbai, India); 5, 5'- dithiobis-2-nitro benzoic acid (DTNB) (Alfa Aesar, A Johnson Mathey Company); bovine serum albumin (Spectrochem Pvt. Ltd., Mumbai, India). All the chemicals used were of analytical grade and purchased from standard manufacturers.

Animals

Wistar strain rats (170-200 g of 9 weeks old) of either sex was used for the study. Animals were procured and housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± 2ºC, 12: 12 h L: D cycle and 50 ± 5% RH with free access to food and water ad libitum. Animals were acclimatized to laboratory conditions before the test. All the experimental work was carried out during the light period (08:00-16:00 h). The study was carried out in harmony with the guidelines given by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi (India). The Institutional Animal Ethical Committee of MVPS, College of Pharmacy, Nashik, India approved the protocol of the study (IEAC/FEB 2023/05).

Induction and assessment of diabetes

16 hours before prior induction of diabetes, the rats were not allowed food although they had access to drinking water. Diabetes was developed in rats by first administering Nicotinamide (NA) (100mg/kg i.p.), following a 15-minute duration. Streptozotocin (STZ) (55mg/kg i.p.) mixed in citrate buffer (0.1M) having a pH of 4.5 was injected to ensure Diabetes. Blood glucose levels were checked after 24, 48 and 72 hours. Rats were restrained and their tails were washed with a warm soap solution and cloth. Under light isoflurane anaesthesia, blood was obtained from the tail vein. Rats exhibiting a glucose level equal to 250 mg/dl or more were chosen for the study. A glucometer (Dr. Morepean) was used to monitor blood sugar levels to confirm hyperglycemia [12].

Experimental design

Here Wistar albino rats were divided into nine groups, each comprising six rats. The experimental design below has been followed for the active phytoconstituent selected for the study. Adult Wistar albino rats weighing 170-200g were used as an experimental model.

Group No.	Group	Drug, dose & route of drug administration
Group I	Normal Control	Distilled water (p.o.)
Group II	Disease Control	Streptozotocin (55mg/kg i.p.) + Nicotinamide (NA) (100 mg/kg i.p.)
Group III	Standard	STZ + NA + Pregabalin (30 mg/kg p.o.)
Group IV	Casticin (low dose)	STZ + NA + CST (7.5 mg/kg p.o)
Group V	Casticin (medium dose)	STZ + NA + CST (15 mg/kg p.o.)
Group VI	Casticin (high dose)	STZ + NA + CST (30 mg/kg p.o.)
Group VII	Cynarin (low dose)	STZ + NA + CYN (5 mg/kg i.p.)
Group VIII	Cynarin (medium dose)	STZ + NA + CYN (10 mg/kg i.p.)
Group IX	Cynarin (high dose)	STZ + NA + CYN (20 mg/kg i.p.)

The behavioural parameters were measured at the end of 1st week, 2nd week, 3rd and 4th weeks of treatment. After 4 weeks, rats were sacrificed by CO_2 euthanasia chamber on the last day, 1 h after all behavioral tests, and sciatic nerves were immediately isolated and tissue homogenate was prepared in 0.1 M Tris–HCl buffer (pH 7.4) for the biochemical and molecular estimations.

Evaluation parameters

Body weight and blood glucose level

Body weight of experimental animals was recorded at the start (Week 0) and end (Week 4) of the study. The blood glucose levels of experimental animals were recorded at the 0th week, 2nd week, 4th week, of the treatment. The blood was collected from tail vein for the estimation of blood glucose levels. A glucometer (Dr. Morepean) was used to monitor blood glucose levels.

Behavioural assessments

Rota Rod Test (Motor Coordination)

This test was done to assess the motor coordination, balance, grip strength and motor learning of animals at rotarod device. Animals with impaired coordination will fall off when the rotation speed exceeds their capacity. Fall of latency is automatically recorded [13].

Actophotometer Test (Locomotor Activity)

The locomotor activity measured using an actophotometer, which operates on photoelectric cells which are connected in circuit with a counter when the beam of light falling on the photocell is cut off by the animal, a count is recorded. Decreased locomotor count was taken as an index of intense peripheral neuropathy. An actophotometer could have either circular or square arena in which the animal moves. The actophotometer contains a square arena (30×30 cm) with walls that are fitted with photocells just above the floor level. The photocells were checked before the beginning of the experiment. The number of times each animal crossed the light beam was recorded automatically for a period of 10 minutes. [14]

Hot Plate Test (Thermal Hyperalgesia)

The Eddy's hot plate test was used to evaluate a condition of altered perception of temperature (thermal hyperalgesia), Operates on the principle of measuring an animal's withdrawal response to a controlled heat stimulus applied to their paws. where a shorter withdrawal time indicates increased pain sensitivity. In this test, animals were individually placed on Eddy's hot plate with the temperature adjusted to 55 ± 1°C. The first sign of jump response or paw licking latency to avoid heat was taken as an index of the pain threshold (the cut-off time was 10s in order to avoid damage to the paw). [15]

Acetone drop test (Cold allodynia)

Cold allodynia was assessed by exposing the injured paw to a cold stimulus (spraying 100 µl of acetone) and observing the response (graded on a 4-point scale) for Fresh drops of 50ul acetone were applied on the mid-plantar surface of the paws gently. The stimulation of cold was created around 2-5 sec of application, and then the responses like mild paw withdrawal, shaking, rubbing, or licking of paw were noted. These reactions were noted as nociceptive responses in terms of minutes on both paws at intervals of 5 minutes and the mean response was noted. [16]

Von Frey Test (Mechanical Allodynia)

The mechanical allodynia was assessed using von Frey filaments (Aesthesio, Samitek Instruments, New Delhi). In brief, the individual rat was placed in a transparent box made from plexiglass having mesh at the bottom. The rat was allowed to acclimatize for 30 min before applying the von Frey filaments. In the context of pain, the sudden withdrawal of a paw or flinching was regarded as a positive response, and failure to withdraw a paw was considered a negative response. First, the filament with 2.0 g force was applied perpendicularly to the ipsilateral (left) hind paw for 3 s until the filament bend. As per the method described by Dixon's up-down, if the rat showed a positive response, a weaker force was applied and if the rat showed a negative response, the filament with increased force (a stronger stimulus) was applied. Each filament was applied thricely keeping a time

interval of 5 min between each application and an average of three applications was used for further analysis [17]

Motor Nerve Conduction Velocity (MNCV)

A nerve conduction velocity (NCV) is an electrical test used to determine nerve impulse conduction down to the nerve that detects nerve injury. These abnormalities in motor nerve conduction may be due to blood flow reduction induced by hyperglycemia. MNCV assessment was done using an 8-channel power lab (AD instruments, Australia) with animal nerve stimulating electrodes and needle electrodes of AD instruments, Australia. Animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg i.p) and Xylazine (10 mg/kg i.p.). Action potential was generated by applying a stimulating electrode at the proximal end and recording was done from the distal end. The distance between the stimulating electrode at the proximal end and the recording electrodes divided by the latent period is calculated as conduction velocity [18]

Biochemical estimation

Tissue homogenate preparation

The animals were sacrificed using a CO_2 euthanasia chamber on the last day, 1 h after all behavioural tests, the portions of the sciatic nerve (from the left legs) were isolated immediately and rinsed in an isotonic saline solution. Then homogenized (10% w/v) with 0.1 M Tris HCl buffer (pH 7.4). The homogenates were kept in ice water for 30 min, followed by centrifugation at 2000×g for 10 min at 4 °C was done and post nuclear fraction was collected for catalase assay (Remi - C30, Remi Industries Ltd. Mumbai, India); for other enzyme assays, centrifugation was at 12000×g for 60 min at 4ºC, was used for subsequent assays. Following biochemical parameters were performed using Microplate reader (BMG LABTECH Spectro star ^{Nano}) [17]

Estimation of catalase (CAT)

Catalase activity was assessed by the method of Luck (1971), where the breakdown of H_2O_2 was measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer (0.0125 M H_2O_2) and 0.05 ml of supernatant of sciatic nerve homogenate and the change in the absorbance was measured at 240 nm. The enzyme activity was calculated using the millimolar extension coefficient of H_2O_2 (0.07). The results were expressed as micro moles of H_2O_2 decomposed per minute per milligram of protein [19]

Estimation of superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of Kono (1978), where in the reduction of nitrobluetetrazolium chloride (NBT) was inhibited by the superoxide dismutase and measured at 560 nm spectrophotometrically. The reaction was started by adding 0.1ml of 1 mM hydroxylamine hydrochloride to a reaction mixture containing 0.1ml of 0.1 mM ethylene diamine tetra acetic acid (EDTA), 0.1 ml of 24 µM NBT, 0.1 ml of 0.03% v/v Triton X 100 reagent, and 1 ml of post nuclear fraction of brain homogenate. After 20 min of incubation at 37℃, the absorbance was measured at 560 nm. The results were expressed at percentage inhibition of reduction of NBT [20]

Estimation of malondialdehyde (Lipid peroxidation assay)

The quantitative measurement of lipid peroxidation in sciatic nerve was done by the method of Wills (1966). By reacting with thiobarbituric acid at 532 nm, this method evaluated the amount of malondialdehyde (MDA) produced. 1.5 ml of 20% acetic acid, 0.1 ml of tissue homogenate, 0.2 ml of 8% sodium lauryl sulphate (SLS), and 1.5 ml of a 0.8% thiobarbituric acid (TBA) solution make up the reaction mixture. Then, the mixture was heated for one hour in a water bath at 95℃. Further, 5 ml of 15:1 n- butanol and pyridine mixture were added. After a vigorous shaking, the mixture was centrifuged for 5 min. The organic layer's upper layer's absorbance was measured at 532 nm. The molar extension coefficient of the chromophore was used to convert the values into nM of MDA per milligrams of protein (1.56 × 105 M-1 cm-1 [21]

Estimation of TNF-α and IL-6 levels in brain: Neuro-inflammatory marker

The quantification of levels of TNF-α and IL-6 in brain were performed by ELISA kit (Krishgen Biosystems, USA) as per instructions of the manufacturer using a microplate reader. The concentrations of TNF-α and IL-6were calculated from standard curves.

Histopathological analysis

The rat sciatic nerve was examined for histopathological evaluation. The sciatic nerve was removed and immediately fixed in 10% buffered formalin. The sciatic nerve, which had been alcohol- dehydrated and embedded in paraffin. Using a microtome, five-micrometer thick serial histological slices were cut from paraffin blocks and stained with hematoxylin and eosin (H&E). Digital microscope (Olympus, Japan), was used to analyses the sections under a light microscope while taking photomicrographs [22]

Statistical analysis

Data was expressed as mean ± standard error mean (SEM). Data analysis was performed using Graph Pad Prism 10.4.1 software (Graph Pad, San Diego, USA). Data of behavioural tests were statistically analyzed using two-way repeated analysis of variance (ANOVA) and Tukey's multiple comparison test was applied for post-hoc analysis, while data of biochemical parameters were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test was applied for post-hoc analysis. A value of $P < 0.0001$ were considered to be statistically significant.

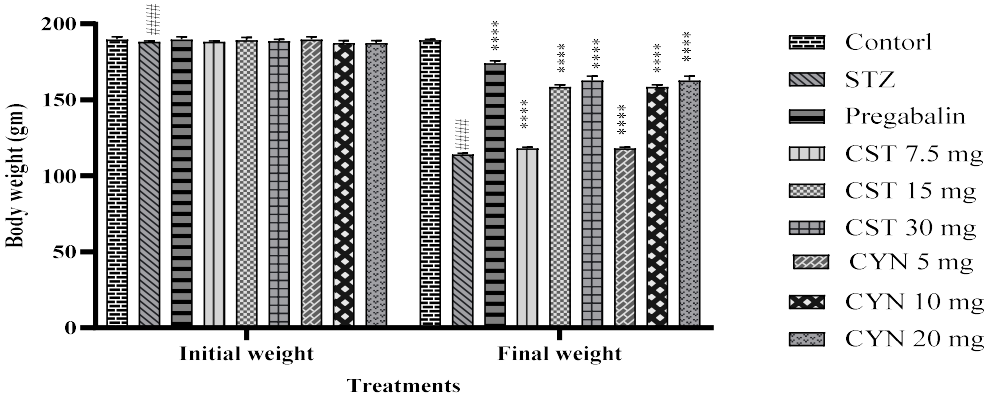
Results

Effect of casticin and cynarin on body weight

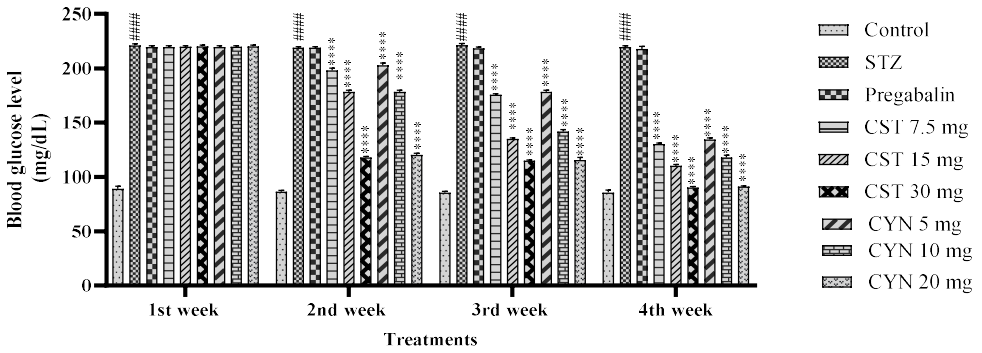
After 72 hrs of STZ treatment, body weight found to be decreased significantly ($p<0.0001^{\#\#\#\#}$) in STZ treated group compared to normal group. Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) treatment have shown significant increase in body weight ($p<0.0001^{****}$) in comparison with positive control group (Fig 1A).

Effect of casticin and cynarin on blood glucose level

After 72 hrs of STZ treatment, blood glucose levels found to be increased significantly ($p<0.0001^{\#\#\#\#}$) in all treatment groups compared to normal group. Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10,20 mg/kg) treatment have shown significant antihyperglycemic effect ($p<0.0001^{****}$) in comparison with positive control group (Fig 1B).



(A)



(B)

Fig.1: Effect of casticin and cynarin on (A) body weight (B) blood glucose level in STZ induced diabetic neuropathy in rats. All values were expressed as mean ± SEM (n=6) and analyzed by two-way ANOVA followed by Tukey's test. *ns*- non significant, $^{\#\#\#\#}P < 0.0001$ as compared to control group, $^{****}P < 0.0001$ as compared to STZ treated group.

Effect of casticin and cynarin on fall of latency in rota rod test

Diabetic rats showed motor in-coordination as indicated by significant ($p<0.0001^{\#\#\#\#}$) decrease in fall latency (sec). Treatment with Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) improvement in motor coordination, indicated by increased fall latency time(sec) than diabetic control rats after 3nd week of treatment schedule (Fig 2A).

Effect of casticin and cynarin on locomotor counts in actophotometer test

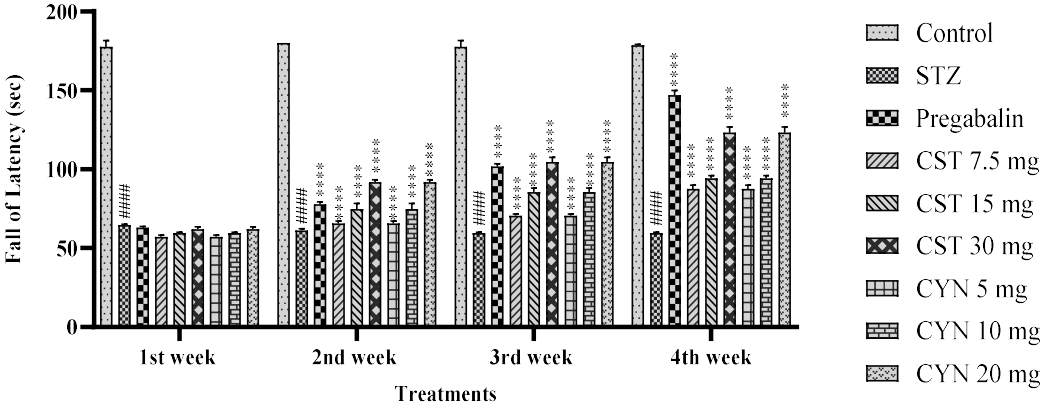
Diabetic rats showed significant ($p<0.0001^{\#\#\#\#}$) decrease in locomotor count as compare to normal group. Treatment with Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) improvement in locomotor counts than diabetic control rats after 3nd week of treatment schedule (Fig 2B).

Effect of casticin and cynarin on paw withdrawal lateny in hot plate test

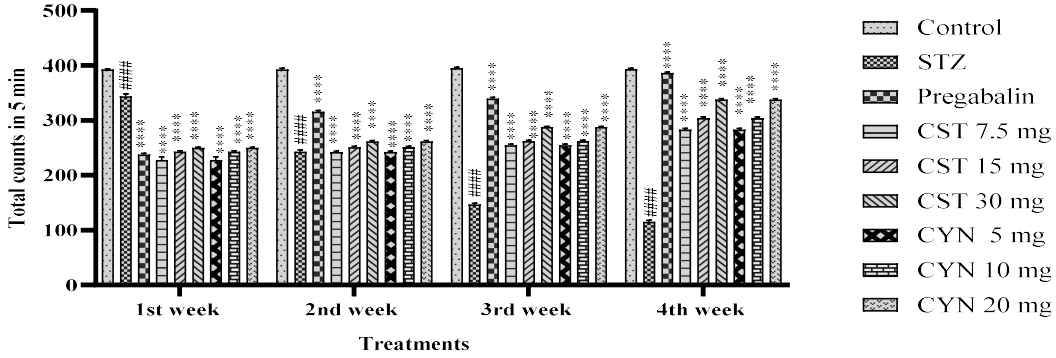
Diabetic rats shown significant reduction ($p<0.0001^{\#\#\#\#}$) in paw withdrawl latency from 2nd week of STZ injection as compare to normal group. Treatment with Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) improvement in paw withdrawl latency at 3rd and 4th week of treatment schedule than positive control rats (Fig 2C).

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

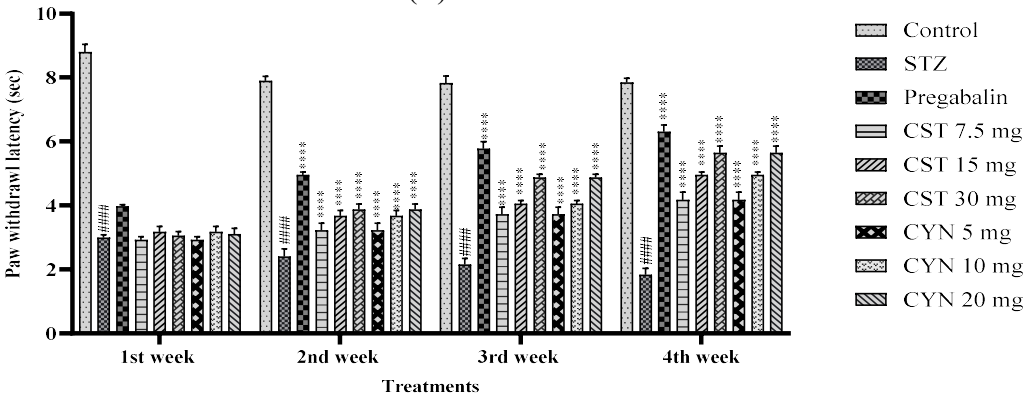
Diabetic rats have shown cold allodynia at the 2nd week of induction of diabetes. Cold allodynia was indicated by decrease of paw withdrawal latency (sec). Diabetic rats shown significant reduction ($p<0.0001^{\#\#\#\#}$) in paw withdrawl latency a 2nd week of STZ injection as compare to normal group. Treatment with Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) improvement in paw withdrawl latency at 3rd and 4th week of treatment schedule than positive control rats (Fig 2D).


Fall of Latency (sec)
200
150
100
50
0
1st week
2nd week
3rd week
4th week
Treatments
Control
STZ
Pregabalin
CST 7.5 mg
CST 15 mg
CST 30 mg
CYN 5 mg
CYN 10 mg
CYN 20 mg

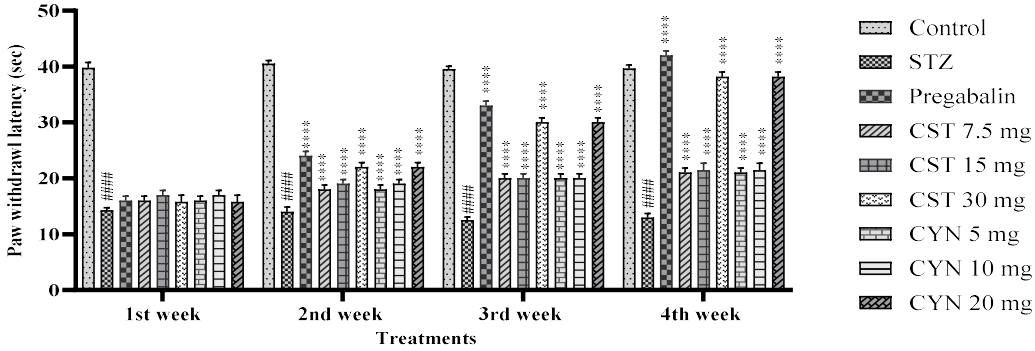
(A)


Total counts in 5 min
500
400
300
200
100
0
1st week
2nd week
3rd week
4th week
Treatments
Control
STZ
Pregabalin
CST 7.5 mg
CST 15 mg
CST 30 mg
CYN 5 mg
CYN 10 mg
CYN 20 mg

(B)


Paw withdrawl latency (sec)
10
8
6
4
2
0
1st week
2nd week
3rd week
4th week
Treatments
Control
STZ
Pregabalin
CST 7.5 mg
CST 15 mg
CST 30 mg
CYN 5 mg
CYN 10 mg
CYN 20 mg

(C)


Paw withdrawl latency (sec)
50
40
30
20
10
0
1st week
2nd week
3rd week
4th week
Treatments
Control
STZ
Pregabalin
CST 7.5 mg
CST 15 mg
CST 30 mg
CYN 5 mg
CYN 10 mg
CYN 20 mg

(D)

Fig.2: Effect of casticin and cynarin on (A) fall of latency (B) locomotor count (C) paw withdrawl latency (D) allodynia score in STZ induced diabetic neuropathy in rats. All values were expressed as mean ± SEM (n=6) and analyzed by two-way ANOVA followed by Tukey's test. *ns*- non significant, $^{\#\#\#\#}P < 0.0001$ as compared to control group, $^{****}P < 0.0001$ as compared to STZ treated group.

Effect of casticin and cynarin on motor nerve conduction velocity (MNCV)

Diabetic rats showed significant ($p<0.0001^{\#\#\#\#}$) reduction in MNCV after 28th day of STZ treatment which indicate nerve damage compared to negative control group. Diabetic rats which are treated Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) improvement in MNCV as compared to diabetic control rats indicating neuroprotective effect (Fig 3).

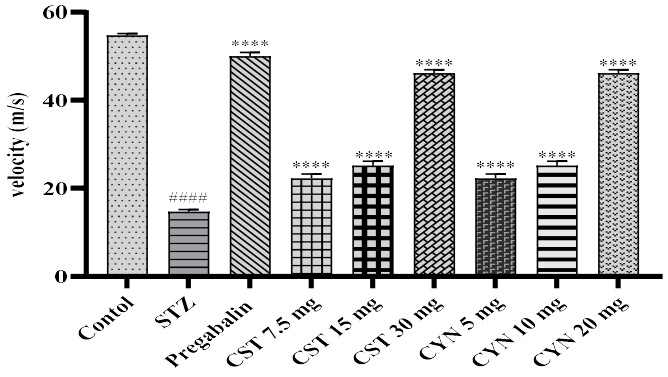


Fig.3: Effect of casticin and cynarin on motor nerve conduction velocity (MNCV) in STZ induced diabetic neuropathy in rats. All values were expressed as mean ± SEM (n=6) and analyzed by two-way ANOVA followed by Tukey's test. *ns*- non significant, $^{\#\#\#\#}P < 0.0001$ as compared to control group, $^{****}P < 0.0001$ as compared to STZ treated group.

Biochemical parameters

All biochemical parameters were examined 1 h after all behavioral tests on last day of week 4 by sacrificing the rats

Effect of casticin and cynarin on catalase (CAT) and Superoxide dismutase (SOD)

In the disease-control animals, CAT, SOD levels were found to be lower compared to those in the Normal Control animals. Results displayed a highly significant ($P<0.0001^{\#\#\#\#}$) reduction in catalase and superoxide dismutase concentration noted in the Disease Control group when compared to normal control group. However, treatment Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) increase in catalase and superoxide dismutase concentration than diabetic rats (Fig 4A, 4B).

Effect of casticin and cynarin on lipid peroxidation (LPO)

A highly significant ($P<0.0001^{\#\#\#\#}$) rise in lipid peroxidation levels was noted in the Disease Control as compare to Normal Control. However, treatment Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) decrease in lipid peroxidation than diabetic rats (Fig 4C).

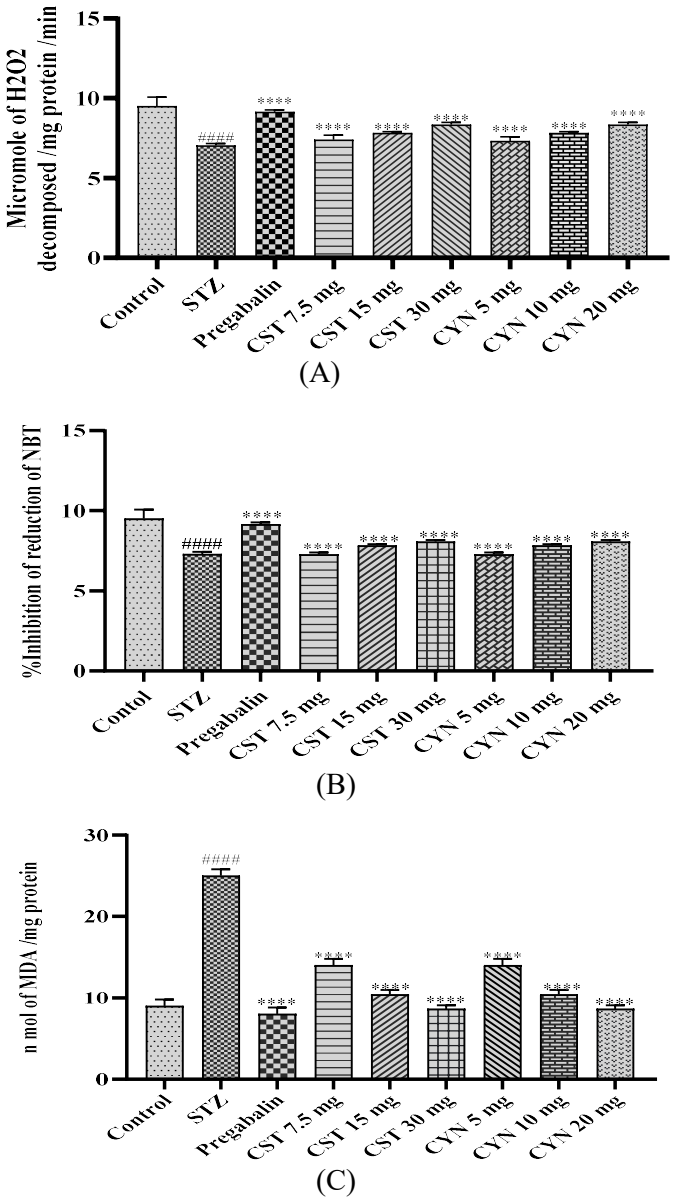


Fig.4: Effect of casticin and cynarin on (A) CAT, (B) SOD, (C) LPO level in STZ induced diabetic neuropathy in rats. All values were expressed as mean ± SEM (n=6) and analyzed by one way ANOVA followed by Tukey's test. *ns*- non significant, $^{\#\#\#\#}P < 0.0001$ as compared to control group, $^{****}P < 0.0001$ as compared to STZ treated group.

Effect of casticin and cynarin on TNF-α and IL-1β levels in rat sciatic nerve

STZ-injected diabetic rats had significantly ($P<0.0001^{\#\#\#\#}$) increased IL-6, TNF-α as compared to normal control rats. However, treatment Pregabalin, Casticin (7.5, 15, 30 mg/kg) and Cynarin (5, 10, 20 mg/kg) showed significant ($p<0.0001^{****}$) decrease in IL-6, TNF- α as compared to diabetic rats. Oxidative stress causes production of abnormal cytokine production (TNF- α, IL-6). Previous studies reported direct inter-relation of TNF- α, IL-1and IL-6 with insulin. Also, it is suggested that inflammation can directly cause insulin resistance. TNF-α decreases insulin secretion by causing inflammation (Fig 5A, 5B).

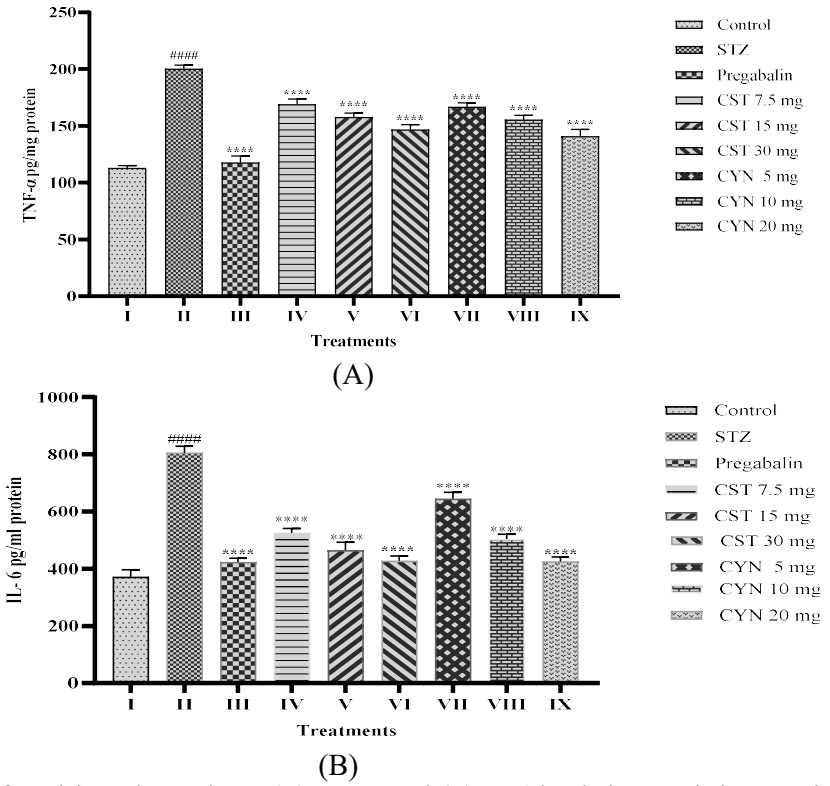


Fig.5: Effect of casticin and cynarin on (A) TNF-α and (B) IL-6 levels in rat sciatic nerve in STZ induced diabetic neuropathy in rats. All values were expressed as mean ± SEM (n=6) and analyzed by one way ANOVA followed by Tukey's test. *ns*- non significant, $^{\#\#\#\#}P < 0.0001$ as compared to control group, $^{****}P < 0.0001$ as compared to STZ treated group.

Histopathological analysis

In histopathological studies it is reported that microscopic examination of sciatic nerve sample from the Normal Control group showed normal neuronal fibers, axon and nuclei. Sciatic nerve from the Disease Control group showed necrosis, neuronal degeneration, vacuolation and inflammation when compared with Normal Control group animals. However, the incidence and severity of these lesions (recorded in Disease Control group) was reduced dose dependently in groups Casticin (7.5, 15, and 30 mg/kg p.o.), and Cynarin (5, 10, and 20 mg/kg i.p.) when compared with Disease Control group suggestive of reversible nature of the change.

Based on these histopathological findings, it can be concluded that animals treated with the test compound, specifically casticin (30 mg/kg) and cynarin (20 mg/ kg) both drugs given orally, exhibited an ameliorative effect and demonstrated significant protective effects on nerve tissue (Fig 5).

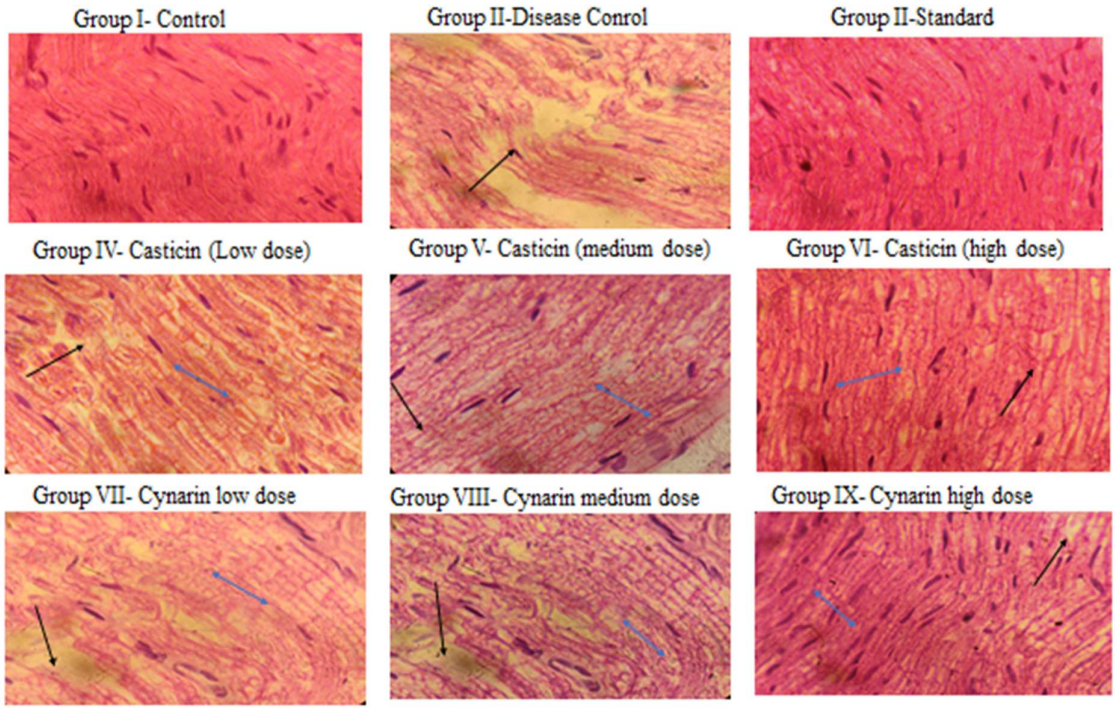


Fig.5: Effect of casticin and cynarin on histology of sciatic nerve of STZ- induced diabetic neuropathy model of rats.

Discussion

This study investigates the effects of Casticin and Cynarin on peripheral neuropathic pain in rats with STZ-induced diabetic neuropathy. The research evaluates various factors such as behavioral changes, biochemical alterations, histopathological observations, and motor nerve conduction velocity (MNCV).

After administering a single injection of STZ (50 mg/kg i.p.) to induce diabetes in rats, all groups showed a significant increase in blood glucose levels (≥ 250 mg/dL) after 72 hours. However, diabetic rats treated with Pregabalin (30 mg/kg p.o.), Casticin (7.5, 15, and 30 mg/kg p.o.), and Cynarin (5, 10, and 20 mg/kg i.p.) displayed a notable reduction in blood glucose levels by the fourth week of treatment, demonstrating Casticin's and Cynarin's potential antihyperglycemic effects.

Diabetic neuropathy is characterized by symptoms such as heat hyperalgesia, cold allodynia, motor incoordination, and mechanical hyperalgesia, all of which are associated with heightened nociceptive response, reduced motor nerve conduction velocity, and neuronal hypoxia. These parameters were evaluated using various behavioral tests, including the hot plate test, cold plate test, rota rod test, open field test, and von Frey hair test.

Behavioral changes indicating the onset of neuropathy were observed in the rats starting from the second week post-diabetes induction. Treatment with Casticin (7.5, 15, and 30 mg/kg p.o.) and Cynarin (5, 10, and 20 mg/kg i.p.) led to significant improvements in these behavioral parameters when compared to the disease control group, suggesting potential therapeutic benefits of these compounds in managing diabetic neuropathy.

Conclusion

In the present study treatment with casticin and cynarin has demonstrated a significant protective effect against STZ-induced diabetic neuropathy. Treatment with Casticin and Cynarin shows significant effect in blood glucose level. This is likely due to their antioxidant level increases, and neuroprotective properties, as evidenced by the reversal of behavioral changes, improvement in histopathological findings, and electrophysiological changes.

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