AMELIORATIVE POTENTIAL OF METHANOLIC TWIGS AND LEAVES EXTRACT OF *NYCTANTHES ARBOR-TRISTIS* ON DIABETES INDUCED NEUROPATHIC PAIN IN ALBINO WISTAR RATS

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ABSTRACT

Background: *Nyctanthes arbor-tristis* is a small ornamental tree renowned for its anti-diabetic activity. Being a rich source of all useful phytoconstituents, traditionally, it's also used in treating many other diseases. However, its role in curing diabetic neuropathy is still not clear. The main objective of this study is to investigate the potential effect of *Nyctanthes arbor-tristis* against streptozotocin (STZ)-induced diabetic neuropathy in rat.

Method: The study was planned with 36 animals and 6 animals in each group. Group 1 (Control group), Group 2 (Diabetic Control), Group 3 (Active Control), Group 4(Test Group-1), Group 5(Test Group-2) & Group-6 (Test Group-3). STZ (50mg/kg) was given intraperitoneally to induce diabetes in Albino wistar rats. After 21 days animals' were assessed for diabetic neuropathy. Rats with diabetic neuropathy were treated for 3 weeks with methanolic extract of *Nyctanthes arbor-tristis* leaves & twigs (100,200,400mg/kg p.o.). Glibenclamide (10mg/kg p.o.) and amitryptiline (10mg/kg i.p.) were used as standard drug. Treatment outcomes were based on metabolic, physiological & biochemical changes.

Result: Treatment with methanolic extract of *Nyctanthes arbor-tristis* significantly decreases blood sugar levels and neuropathic pain as compared to the disease control

group. The effect of MENA (400mg) was observed to be better than the effect of MENA (100mg and 200mg) and comparable to a standard group while the effect of 100mg was significantly lesser than that of all the other treated groups.

Conclusion: Nyctanthes arbor-tritis leaves and twigs, being a potent antioxidant has shown positive results in treating hypoglycemia and diabetic neuropathy.

Keywords: Diabetic neuropathy, Streptozotocin, Glibenclamide, Antioxidant

1. Introduction

Diabetes mellitus is a well-known metabolic condition that is characterised by hyperglycemia and other metabolic problems brought on by abnormalities in insulin secretion or action. A rising corpus of scientific research demonstrates a connection between some neurodegenerative illnesses and metabolic problems. Epidemiologic studies have shown that having diabetes significantly increases the likelihood of developing neural tissue damage and that it has significant effects on both the peripheral and central nervous systems.[1,2]

The fundamental underlying cause of damage to the neurological system in diabetic patients, hyperglycemia, is widely known. According to some research findings, diabetic neuropathy is linked to the ongoing production of reactive oxygen species through glucose auto-oxidation, which results in the development of advanced glycation end-products, activation of the nuclear enzyme polyADP-ribose polymerase, and a decrease in antioxidant defence.[3,4]

A small ornamental tree named *Nyctanthes arbor-tristis* Linn. (NAT) of family oleaceae, is well-known throughout the nation for its scent and white blossoms and as a traditional herb which has shown successful results as antidiabetic, antimicrobial, hepatoprotective, immunomodulatory & anti-depressant activity.

Phyto constituents like steroids (β- sitosterol, Astragaline, Nicotiflorin, Nyctanthic acid), Alkaloids (Nyctanthine), Glycosides (astragaline, nicotiflorine, arbortristoside C, nyctanthoside), Flavonoids (Apigenin, quercetin, kaempferol, luteolin), Triterpenoids(oleanolic acid, friedeline, lupeol) are present in Nyctanthes arbortritis which are responsible for various pharmacological activity.[5,6]

These constituents play a vital role in one such activity that is hyperglycemia. Elevated hyperglycemia leads to risk factor of neuropathy by hyperactivtion of:

i. Polyol pathway

ii. Hexoamine pathway

iii. Protein kinase C pathway

iv. Advanced glycation end product pathway[7,8,9]

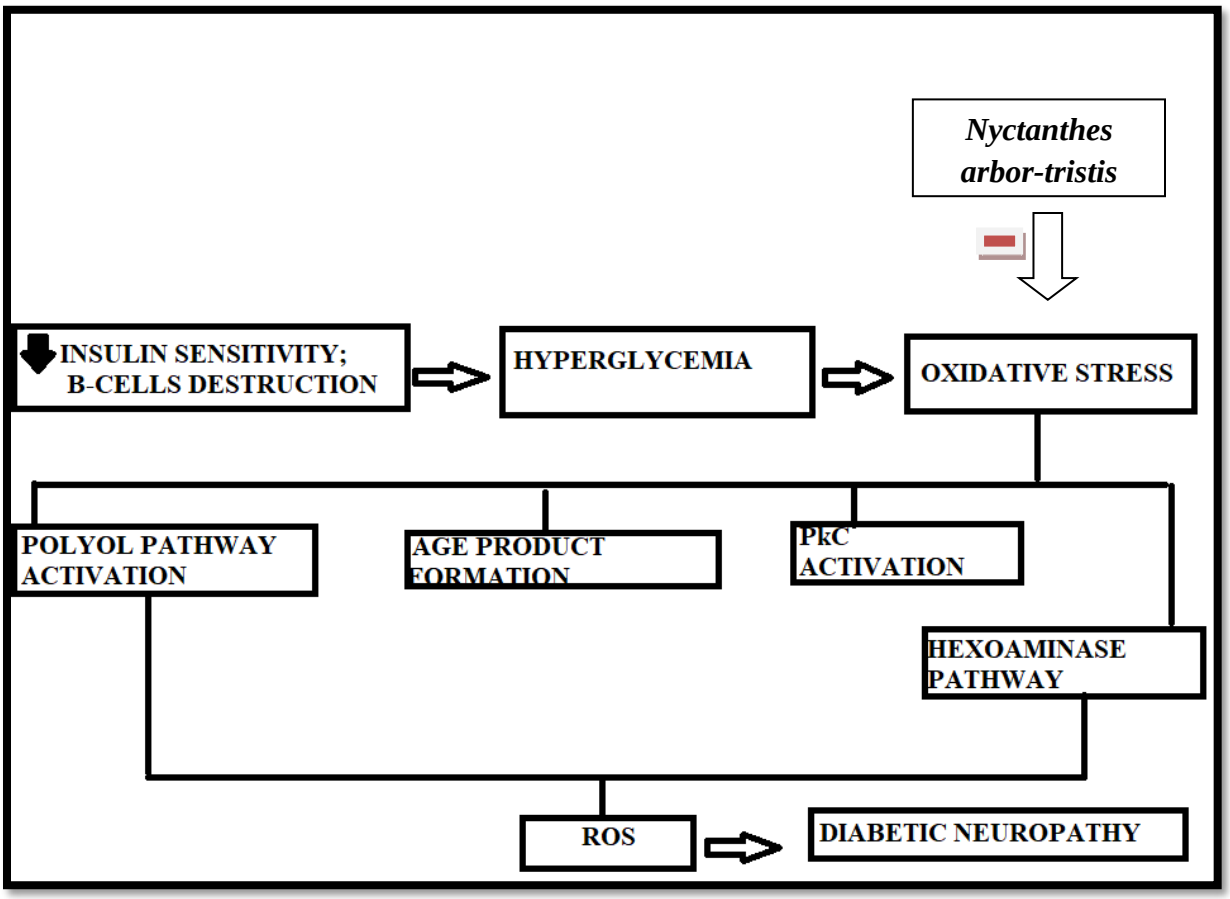

Fig.1 Oxidative stress leading to Diabetic Neuropathy

The impact of *Nyctanthes arbortritis* on STZ-induced diabetic neuropathy should therefore be studied. The findings of this study will significantly benefit the underprivileged and those living in rural areas, which tend to prefer herbal treatments for diabetes that are less expensive than conventional ones.

Fig.2 *Nyctanthes arbor-tristis* tree

2. Material & methods

2.1 Plant material

The plant was collected from Herbal garden of Shri Guru Ram Rai University, Dehradun and will be identified by Botanical Survey of India (BSI), Dehradun.

2.2 Preparation of methanolic extract: Fresh leaves were taken from the plant, carefully washed, and dried in the shade. Using a mechanical grinder, the dried leaves were reduced to a coarse powder. A rotatory shaker was used to sometimes agitate 200 g of the coarse powder material for 4 days while it was soaked in 500 ml of methanol. The extracts were concentrated using a rotary evaporator at 40°C until completely dry. The filtrate was then passed through Whatman filter paper (125 mm).[10]

Fig.3 Methanolic extract of *Nyctanthes arbor-tristis* by maceration

2.3 Drugs, Chemicals & Kits

All the chemicals, drugs (STZ, glibenclamide,amitryptiline) and GOD-POD kit were all provided by Shri Guru Ram Rai University, Patel nagar, Dehradun.

2.4 Preliminary Phytochemical Screening[11, 12]

Table 1. Phytochemical screening

Phytochemical Test	**Name of the Test**	**Methanolic extract**
Alkaloids	Wagner's test	**+**
Flavonoids	Alkaline reagents	+
Glycosides	Keller-Killani test	+
Saponins	Froth test	+

Steroids / Terpenoids	Salkowski's test	+
Tannins	Ferric chloride test	+

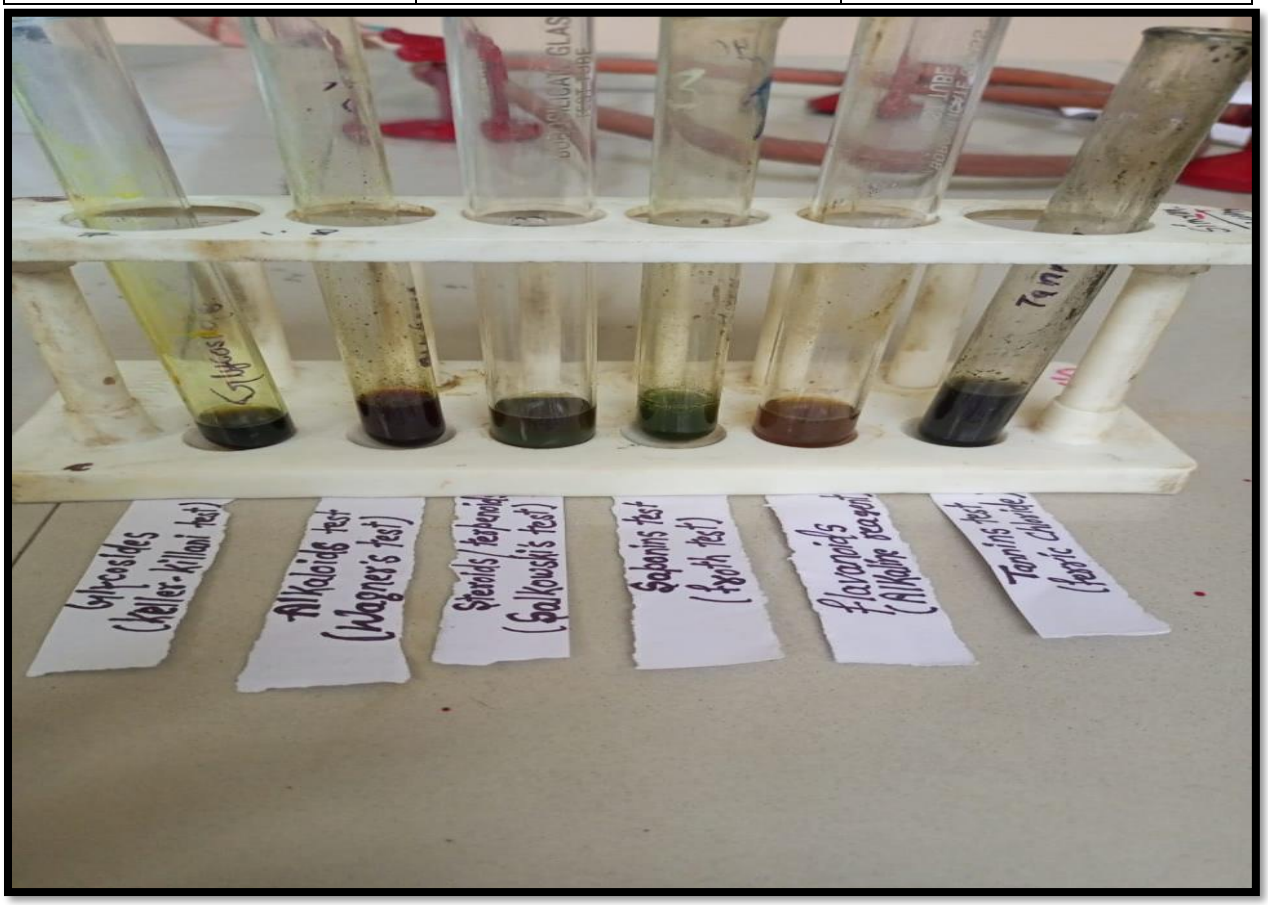

Fig.4 Phytochemical Screening

2.5 Animals

Albino Wistar rats (120-150gm) of either sex (6-8 weeks of age) were procured from the departmental animal house of Shri Guru Ram Rai University, Dehradun. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) and executed according to guidelines of CPCSEA with No.: **264/CPCSEA/IAEC/2022/04.**

Housing Conditions

Under typical laboratory circumstances, the animals were kept in polypropylene cages with husk bedding at a temperature of 22 ± 20 °C and a humidity of 50 ± 15 %, with a cycle of 12 hours of day and 12 hours of night. The animals were given a standard laboratory meal and given unlimited access to water. All protocols were performed following the Institutional Animal Ethical Committee (IAEC) as per the directions of the CCSEA (Committee for the Control and Supervision of Experiments on Animals).

2.6 Induction & assessment of Diabetes

In order to induce diabetes, rats were kept on an overnight fast before receiving a single injection of STZ (50 mg/kg i.p.) dissolved in freshly made citrate buffer (pH 4.5, 0.1M). Normal saline was given to the animals used as control. Rats were given 5% w/v glucose solution over the first 24 hours to help them recover from hypoglycemic shock. Seventy-two hours later, blood samples were collected from the tail vein to assess blood glucose levels. The measurements were made using a Glucometer. The study included rats with type 1 diabetes who had fasting blood glucose levels greater than 250 mg/dL..[13,14]

Rats were randomly assigned into the following groups

6 animals in each group were randomly selected and divided into 6 groups:

GROUPS	**TREATMENT**
Group 1 (Control group)	10 ml/kg normal saline p.o. was administered as vehicle.
Group 2 (Diabetic control)	STZ (50 mg/kg, i.p.) was administered for induction of diabetes.
Group 3 (Active control)	STZ (50mg/kg, i.p) once + Glibenclamide (10mg, p.o) + Amitryptline(10mg/kg, i.p) for 21 days.
Group 4 (Test group-1)	Diabetic rats (STZ 50 mg/kg, i.p) + 100mg/kg/day of extract for 21 days.

Group 5 (Test group-2)	Diabetic rats (STZ 50 mg/kg, i.p) + 200mg/kg/day of extract for 21 days.
Group 6 (Test group-3)	Diabetic rats (STZ 50 mg/kg, i.p) + 400mg/kg/day of extract for 21 days.

2.7 Parameters evaluated

2.7.1 Behavioural Parameters

2.7.1.1 Thermal Allodynia (Eddy's Hot Plate Method):

The rotarod treadmill test was used to evaluate the rats' motor coordination. On day 0 and the last day of the investigation, rats were used in two trials using the rotating rod apparatus. They were trained to remain stable on the revolving rod for more than three minutes, and after that, each trial's falling latency (measured in seconds) was scored.[15]

2.7.1.2 Thermal Hyperalgesia (Tail Immersion Method):

The animals were given a 30-minute acclimation period. The test animal's tail was immersed in warm water that was maintained at a temperature of 55± 0.5℃.The amount of time it takes an animal to remove its tail from hot water is known as the thermal pain response. The duration of each animal's response to thermal discomfort was noted. The tail-to-hot-water cutoff interval was 15 seconds in order to avoid tissue damage.[16, 17]

2.7.1.3 Hot-plate method

Animals were placed individually on pre-heated platforms at 55± 0.5 ℃ of hot plate after receiving *Nyctanthes* treatment for 21 days. Animals were closely watched for signs of thermal pain, such as licking, flashing, and jumping. Each animal's first response, whether it was a flick, lick of the rear paw, or a jump, was timed. To prevent causing tissue injury, the cutoff time was 15s.[18, 19]

2.7.2 Biochemical estimation

2.7.2.1 Determination of serum glucose level

The commercially available enzymatic GOD-POD kit, or glucose oxidase/peroxidase diagnostic kit, was used to measure the levels of serum glucose.

2.7.2.2 Determination of SOD

The Paoletti and Mocali method was used to calculate the SOD concentration. The basic idea behind this approach is the oxidation of NADH, which is carried out in a purely chemical system and is mediated by superoxide radical. Through a free-radical chain of events involving thiol oxidation and univalent reduction, coenzyme oxidation happens in the presence of appropriate concentrations of EDTA, Mn^{2+}, and mercaptoethanol. The rate of NADH oxidation is significantly inhibited when SOD is added to the reaction mixture, demonstrating the role of superoxide in the reaction and establishing the methodology for measuring SOD activity. After adding mercaptoethanol, a drop in absorbance at 340 nm was seen for around 15 minutes.[20,21,22]

2.7.2.3 Determination of CAT

The method described by Luck was used to measure the amount of catalase. UV absorption of H2O2 solution's can be precisely measured between 230 and 250μ. The absorption diminishes over time as H2O2 is broken down by catalase, and from this decline, the enzyme activity can be determined. Only enzyme solutions with weak 230–250 μ absorption can be used with this technique.[23, 24]

2.8 Statistical Analysis

All results were expressed as mean ± standard error of mean (SEM). The statistical analysis was carried out using Graph pad prism 9.5.0. Data were analysed by applying one way analysis of variance (ANOVA) followed by Tukey's test $p \leq 0.05$ was considered to be statistically significant.

2.9 Results

2.9.1 Effect of MENA on Thermal allodynia (Eddy's hot plate) in rats

Administration of STZ significantly ($p<0.001$) decreases paw withdrawal latency as compared to the control group. However, treatment with MENA (100 mg, 200mg, 400mg) significantly increases the paw withdrawal latency ($p<0.05$ and $p<0.01$) respectively as compared to disease control group (STZ). The effect of MENA (400 mg) ($p<0.01$) was observed to be better than

MENA (200 mg) ($p<0.05$) while the effect of MENA 100 mg was significantly lesser than all the other treated groups.

Table 2: Effect of MENA on Thermal allodynia (Eddy's hot plate) in rats:

Thermal allodynia (Eddy's hot plate) mean ± SEM			
Groups	**Day 7**	**Day 14**	**Day 21**
Control	4.19±1.29	4.12±1.43	4.01±1.03
STZ 50 mg/kg i.p.	6.67±1.28^{a**}	8.23±2.92^{a**}	9.85±1.86^{a**}
Glib10 g/kg, p.o. + Ami. 10 mg/kg, i.p.	5.30±4.19^{b*}	5.89±1.76^{b*}	5.91±1.74 ^{b*}
MENA 100 mg/kg, p.o.	6.92±0.29$^{c,\ d**}$	6.55±1.32$^{c.\ d**}$	6.08±1.41$^{c,\ d**}$
MENA 200 mg/kg, p.o.	6.01±0.29$^{c\#,\ d***}$	5.93±0.24$^{c\#,\ d***}$	5.15±1.27$^{c\#,\ d***}$
MENA 400 mg/kg, p.o.	5.85±0.26$^{c*,\ d\#}$	5.16±1.82$^{c*,\ d\#}$	5.11±1.65$^{c*,\ d\#}$

*Data are expressed as mean ± SEM, (n=6), #p<0.05,*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control and disease control (STZ), analyzed by one way ANOVA followed by Tukey's posthoc test.*

a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between STZ (disease control group) and MENA (100mg, 200 mg, 400 mg/kg) (Test group)

(d) Represents significant difference between Glibenclamide + Amitryptiline (standard group) and MENA (100mg, 200 mg, 400mg) (Test group)

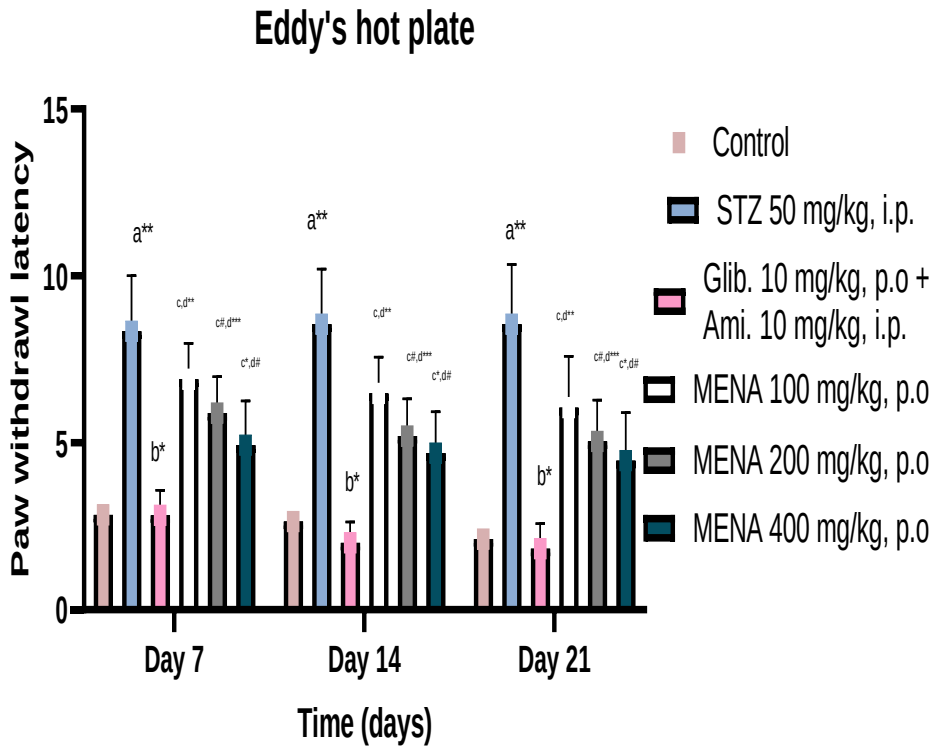

Fig.5: Effect of MENA on Thermal allodynia (Eddy's hot plate) in rats

2.9.2 Effect of MENA on Thermal hyperalgesia (Tail immersion) in rats:

Administration of STZ significantly ($p<0.05$) decreases tail withdrawal latency as compared to the control group. However, treatment with MENA (100 mg, 200mg, and 400mg) significantly increases the tail withdrawal latency as compared to disease control group (STZ). The effect of MENA (400 mg/kg) was observed to be better than MENA (200 mg/kg) while the effect of MENA 100 mg/kg was significantly lesser than all the other treated groups.

Table 3: Effect of MENA on Thermal hyperalgesia (Tail immersion) in rats:

Thermal hyperaalgesia (Tail Immersion) (in sec) mean ± SEM			
Groups	**Day 7**	**Day 14**	**Day 21**
Control	3.67±2.15	3.43±3.20	3.08±2.20
STZ 50 mg/kg i.p.	7.02±2.16[a#]	7.90±1.52[a#]	8.05±1.78[a#]
Glib10 g/kg, p.o. + Ami. 10 mg/kg, i.p.	2.83±0.75[b#]	2.00±0.63[b#]	1.83±0.75[b#]
MENA 100 mg/kg, p.o.	7.07±3.38[c, d**]	6.81±3.93[c, d**]	6.03±2.18[c, d**]
MENA 200 mg/kg, p.o.	5.84±2.67[c, d*]	5.63±2.07[c, d*]	5.43±2.69[c, d*]
MENA 400 mg/kg, p.o.	4.50±0.54[c, d#]	4.00±0.63[c, d#]	3.33±1.03[c, d#]

*Data are expressed as mean ± SEM, (n=6), #p<0.05,*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control and disease control (STZ), analyzed by one way ANOVA followed by Tukey's posthoc test.*

a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between STZ (disease control group) and MENA (100mg, 200 mg, 400 mg/kg) (Test group)

(d) Represents significant difference between Glibenclamide + Amitryptiline (standard group) and MENA (100mg, 200 mg, 400mg) (Test group)

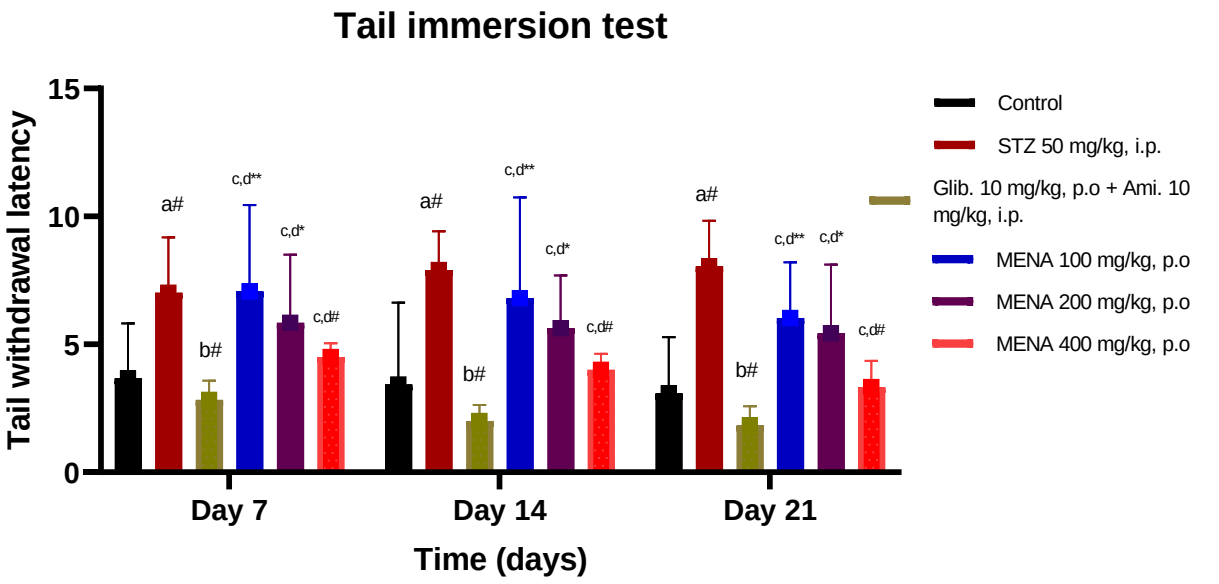

Fig. 6: Effect of MENA on Thermal hyperalgesia (Tail immersion) in rats

2.9.3 Effect of MENA on grip strength in rats:

Administration of STZ significantly ($p<0.0001$) decreases grip strength as compared to the control group. However, treatment with MENA (100 mg, 200mg, 400mg) significantly increases the grip strength ($p<0.01$, $p<0.0001$ and $p<0.001$) respectively as compared to disease control group (STZ). The effect of MENA (400 mg) ($p<0.01$) was observed to be better than MENA (200 mg) ($p<0.0001$) while the effect of MENA 100 mg ($p<0.001$) was significantly lesser than all the other treated groups.

Table 4: Effect of MENA on grip strength in albino wistar rats:

Grip strength (in sec) mean ± SEM			
Groups	**Day 7**	**Day 14**	**Day 21**
Control	32.55±2.05	32.5±2.05	32.64±2.06
STZ 50 mg/kg i.p.	8.82±0.55^{a***}	8.13±0.51^{a***}	8.32±0.52^{a***}
Glib10 g/kg, p.o. + Ami. 10 mg/kg, i.p.	27.33±1.72^{b*}	28.86±1.82^{b*}	30.77±1.94^{b*}
MENA 100 mg/kg, p.o.	21.43±1.35$^{c**, d*}$	22.58±1.42$^{c**, d*}$	23.11±1.46$^{c**, d*}$
MENA 200 mg/kg, p.o.	23.69±1.49$^{c***, d}$	23.98±1.51$^{c***, d}$	24.20±1.52$^{c***, d}$
MENA 400 mg/kg, p.o.	24.86±1.57$^{c*, d}$	25.01±1.67$^{c*, d}$	26.31±1.66$^{c*, d}$

*Data are expressed as mean ± SEM, (n=6),*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control and disease control (STZ), analyzed by one way ANOVA followed by Tukey's posthoc test.*

a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between STZ (disease control group) and MENA (100mg, 200 mg, 400 mg/kg) (Test group)

(d) Represents significant difference between Glibenclamide + Amitryptiline (standard group) and MENA (100mg, 200 mg, 400mg) (Test group)

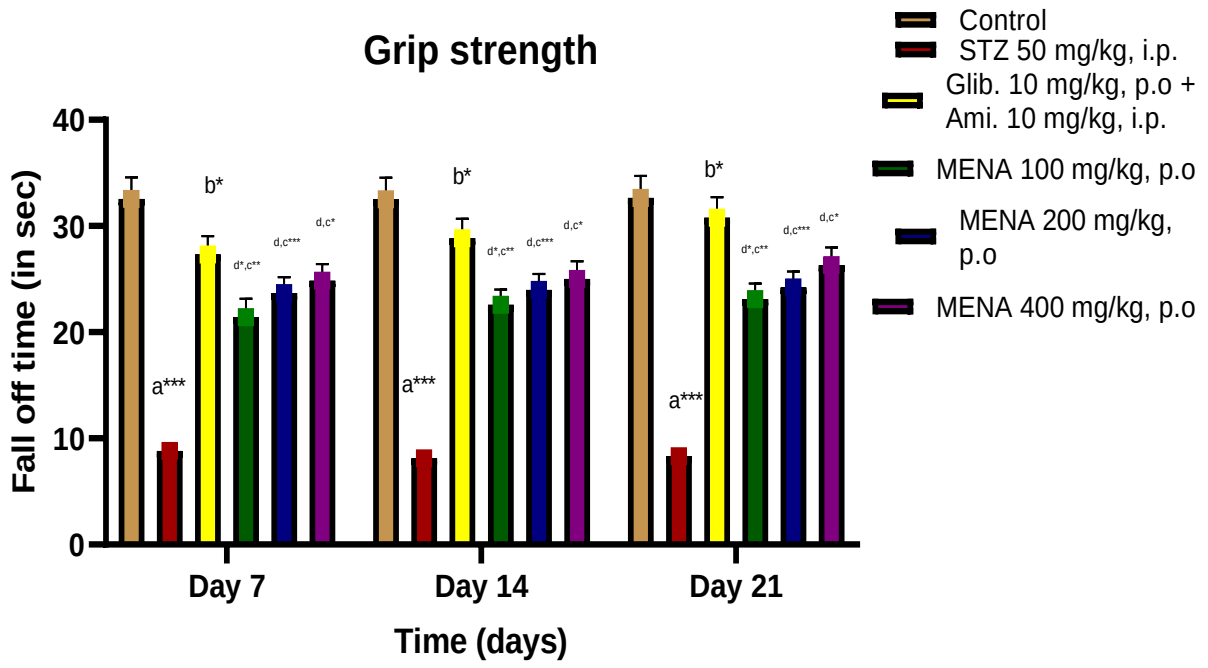

Fig 8: Effect of MENA on grip strength in rats

2.9.4: Effect of STZ on serum glucose level in rats:

Serum glucose level was estimated on 0, 7th, 14th and 21st days in all STZ treated groups and the result was found significant in comparison to the control group. The diabetic rats were selected more than 250 mg/dl after administration of STZ.

Table 5: Effect of STZ on serum glucose level in rats:

Blood glucose level(mg/dl) mean±SEM				
Groups	**Day 0**	**Day 7**	**Day 14**	**Day 21**
Control	83±5.24	83±5.24	84±5.31	86±5.43
Diabetic control	95.88±6.06	188±11.89	218±13.78	250±15.81

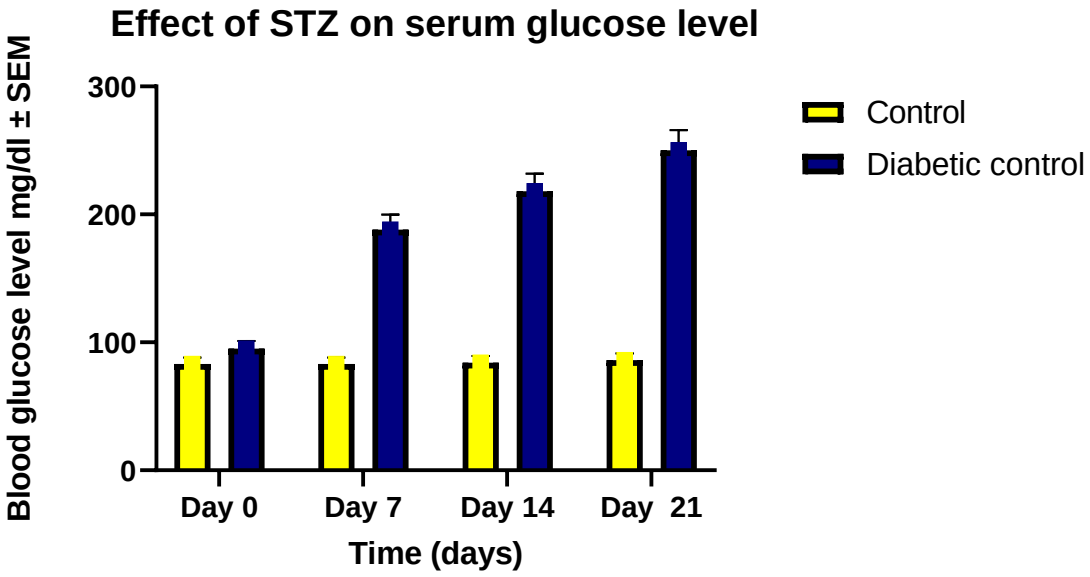

Fig 9: Effect of STZ on serum glucose level in rats.

Here, STZ represents Streptozotocin. All values represented as mean ± SEM (n=6). Data were compared by using one way ANOVA followed by Tukeys post test.

2.9.5 Effect of MENA on fasting serum glucose level in rats:

Administration of STZ significantly ($p<0.0001$) increases blood sugar level as compared to the control group. However, treatment with MENA (100mg, 200mg, 400mg) significantly ($p<0.0001$) decreases blood sugar level as compared to the disease control group (STZ). The effect of MENA (400 mg) was observed to be better than the effect of MENA (100mg and 200 mg) while the effect of 100 mg was significantly lesser than that of all the other treated groups.

Table 6: Effect of MENA on fasting serum glucose level in rats:

Fasting serum glucose level (mg/dl) mean ± SEM			
Groups	**Day 7**	**Day 14**	**Day 21**
Control	84.5 ±4.20	85±5.37	84±5.31
STZ 50 mg/kg i.p.	275±7.18^{a***}	280±17.70^{a***}	286±18.08^{a***}
Glib10 mg/kg, p.o. + Ami. 10 mg/kg, i.p.	85.61±4.21^{b***}	85±5.43^{b***}	86±5.37^{b***}
MENA 100 mg/kg, p.o.	101.2±1.91$^{c***, d**}$	101±6.38$^{c***, d**}$	100±6.32$^{c***, d**}$
MENA 200 mg/kg, p.o.	96.5±2.26$^{c***, d*}$	96±6.07$^{c***, d*}$	95±6.00$^{c***, d*}$
MENA 400 mg/kg, p.o.	88.5±3.19$^{c***, d}$	88±3.56$^{c*** , d}$	87±5.50$^{c***, d}$

*Data are expressed as mean ± SEM, (n=6),*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control and disease control (STZ), analyzed by one way ANOVA followed by Tukey's posthoc test.*

a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between STZ (disease control group) and MENA (100mg, 2000 mg , 400 mg/kg) (Test group)

(d) Represents significant difference between Glibenclamide + Amitryptiline (standard group) and MENA (100mg, 200 mg, 400mg) (Test group)

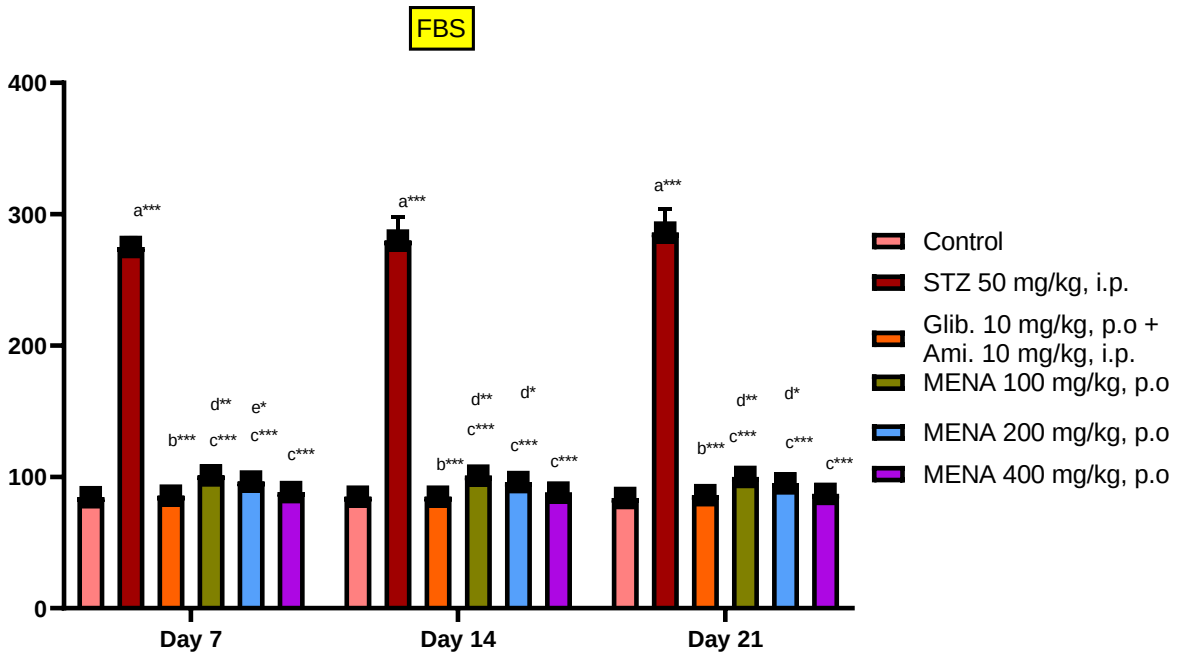

Fig 10: Effect of MENA on fasting serum glucose level in rats

2.9.6 : Effect of MENA on level of SOD in rats:

Administration of STZ significantly ($p<0.0001$) decreases level of SOD as compared to the control group. However, treatment with MENA (100 mg, 200mg, 400mg) significantly increases SOD levels ($p<0.001$ and $p<0.0001$) respectively as compared to disease control group (STZ). The effect of MENA (400 mg) ($p<0.0001$) was observed to be better than MENA (200 mg) ($p<0.001$) while the effect of MENA 100 mg was significantly lesser than all the other treated groups.

Table 7: Effect of MENA on level of SOD in rats:

Groups	**Level of SOD(unit/mg of protein) ± SEM**
Control	18.83±1.19
STZ 50 mg/kg i.p.	$5.45\pm0.34^{a***}$
Glib10 g/kg, p.o. + Ami. 10 mg/kg, i.p.	$17.05\pm1.07^{b***}$
MENA 100 mg/kg, p.o.	$8.41\pm0.53^{c, d***}$
MENA 200 mg/kg, p.o.	$9.87\pm0.62^{c**, d***}$
MENA 400 mg/kg, p.o.	$12.72\pm1.05^{c***, d**}$

*Data are expressed as mean ± SEM, (n=6),*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control, disease control (STZ) and standard, analyzed by one way ANOVA followed by Tukey's posthoc test.*

(a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between STZ (disease control group) and MENA (100mg, 200 mg, 400 mg/kg) (Test group)

(d) Represents significant difference between Glibenclamide + Amitryptiline(standard group) and MENA (100mg, 200mg, 400mg) (Test group)

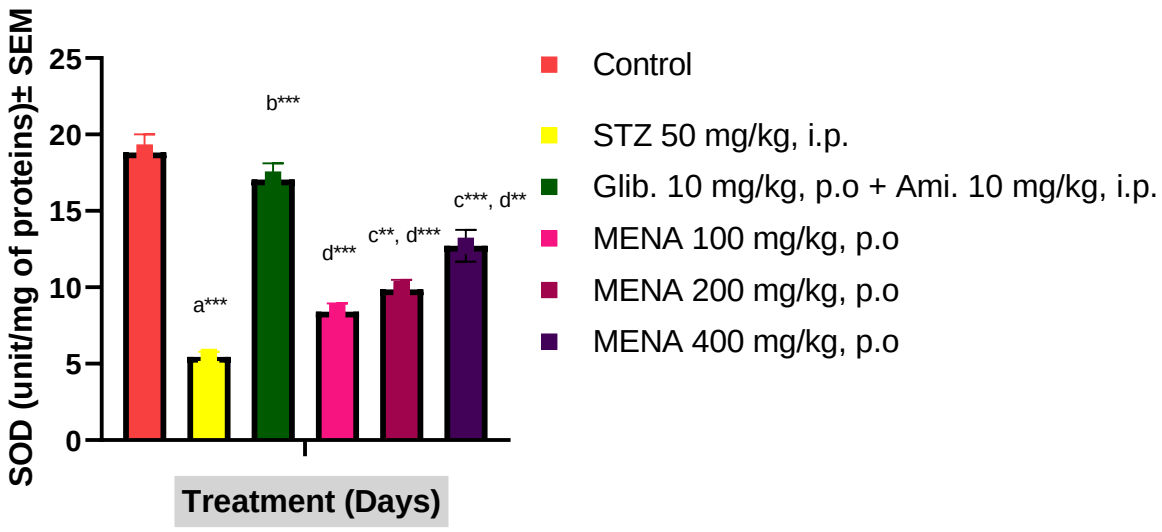

Fig 11: Effect of MENA on level of SOD in rats

2.9.7 Effect of MENA on Catalase (CAT) level in STZ induced diabetic Albino wistar rats:

Administration of STZ significantly ($p<0.0001$) decreases level of CAT as compared to the control group. However, treatment with MENA (100mg, 200mg, and 400mg) significantly increases CAT level as compared to disease control group (STZ). The effect of MENA (400mg) was observed to be better than effect of MENA (200 mg) while MENA (100mg) was significantly lesser than other treated groups.

Table 8: Effect of MENA on level of CAT in rats:

Groups	**Level of CAT(unit/mg of protein) ± SEM**
Control	16.58±1.20
STZ 50 mg/kg i.p.	$4.72\pm1.21^{a***}$
Glib10 g/kg, p.o. + Ami. 10 mg/kg, i.p.	$15.67\pm2.42^{b**}$
MENA 100 mg/kg, p.o.	$6.43\pm1.91^{c*}$
MENA 200 mg/kg, p.o.	$7.80\pm2.26^{c*}$
MENA 400 mg/kg, p.o.	9.08 ± 1.19^{c}

*Data are expressed as mean ± SEM, (n=6),*p<0.01, **p<0.001 and ***p<0.0001 significantly different as compared to control, disease control (STZ) and standard, analyzed by one way ANOVA followed by Tukey's posthoc test.*

(a) Represents significant difference between STZ (disease control group) and Control

(b) Represents significant difference between STZ (disease control group) and Glibenclamide + Amitryptiline (standard group)

(c) Represents significant difference between Glibenclamide + Amitryptiline(standard group) and MENA (100mg, 200mg,400mg) (Test drug)

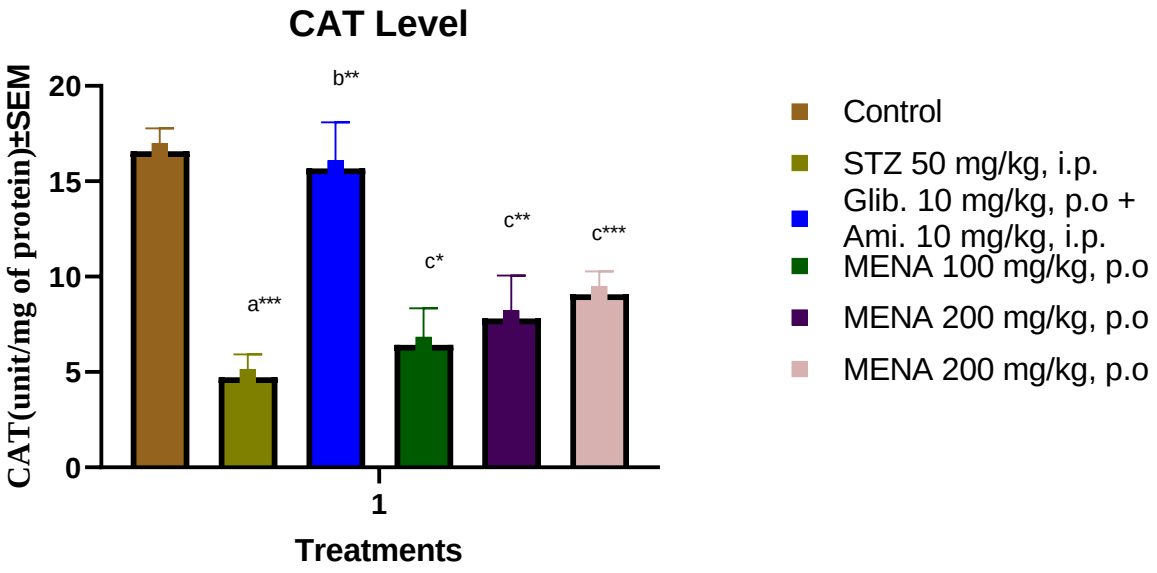

Fig 12: Effect of MENA on level of CAT in rats

3.0 DISCUSSION

A sizable portion of the global population is impacted by hyperglycemia and diabetic neuropathy, the two most prevalent complications of diabetes. Both inflammation and hyperglycemia have an effect on gene expression, which in turn has an effect on cellular proteins and results in gradual modifications, such as pathological abnormalities and consequences from diabetes. Under the present therapeutic paradigm, antidepressant drugs have been used to treat and control neuropathic pain, which results in the maintenance of sustained levels of neurotransmitters (norepinephrine and serotonin).[25, 26]

Given the shortcomings of existing therapy for DPN, additional research is required to determine and develop the most effective treatment regimen for diabetic neuropathy.

According to published research, plants having antioxidant activity can treat neuropathy. The phytochemistry of the leaves and twigs of Nyctanthes arbortristis has revealed the presence of several potentially bioactive antioxidants, including Ascorbic Acid, Iridoid Glycosides, Quercetin, Lupeol, Nyctanthic Acid, Oleanolic Acid, -Sitosterol, and Arbortristoside A and B. Diverse plant parts have been found to offer a wide range of pharmacological actions, such as

anti-diabetes, anti-oxidant, anti-inflammatory, anti-microbial, anti-pyretic, analgesic, immunomodulatory, CNS modulatory, etc.[27, 28, 29, 30]

The current work has investigated the effects of NAT at doses of 100, 200, and 400 mg/kg on streptozotocin-induced diabetes and diabetic neuropathy in Albino Wistar rats. Additionally, tests for nociception, response latency, neuromuscular coordination, and biochemical parameters such serum glucose level and SOD and CAT determination were carried out.

A glucosamine-nitrosourea called STZ is made from the bacterium Streptomyces achromogenes. Animal models for diabetes and its consequences, including diabetic peripheral neuropathy, are created using STZ as an experimental tool. The pathology of diabetic neuropathy has been linked to increased advanced glycation end products (AGEs), receptors for AGEs (RAGE), and free radical-induced oxidative stress. Therefore, in the current investigation, rats were given a single dosage of STZ (50 mg/kg i.p.) to create a diabetic neuropathic pain model of allodynia.[31,32]

100, 200, and 400 mg/kg NAT dosages resulted in a reduction in blood glucose levels. All three of the NAT doses worked to lower blood sugar levels, but the 400 mg/kg dose had the most impact; nonetheless, it was not as successful as the glibenclamide standard dose of 10 mg/kg i.p.

Due to the advancement of discomfort and the onset of diabetic neuropathy, animals that were beginning to suffer from nerve damage have a tendency to become lethargic and exhibit delayed reactions. The results of thermal allodynia, thermal hyperalgesia, and motor coordination in diabetic rats also revealed a loss of feeling of hot or cold pain due to severe nerve damage, and better outcomes in the treated groups due to their ability to mend nerve damage.[33,34]

The study found that diabetic disease group (STZ) animals responded more slowly because of significant nerve damage and loss of perception brought on by long-term diabetes. Treatment groups experienced a progressive healing response, which was most pronounced in those receiving NAT doses of 200 mg/kg and 400 mg/kg. In comparison, the diabetic groups have shown a trend of high sensitivity responses in nerves to delayed responses due to extensive nerve damage but the results were not better than that of standard group which was treated with Glibenclamide (10 mg/kg p.o.) and Amitryptiline (10 mg/kg i.p.).

Methanolic extract of NAT possess various antioxidant, tannins and alkaloids which is why its administration in different doses i.e. 100 mg/kg, 200 mg/kg and 400 mg/kg was able to bring about the change in the concentration of SOD and CAT as compared to diabetic control group. Effective dose was found to be 400 mg/kg and then 200 mg/kg. Dose of 100 mg/kg has also shown change in the concentration of SOD and CAT but so far the best results were found at the dose of 400 mg/kg.

The current study showed scientific evidence for attenuation of oxidative stress, prevention of nerve damage via improving the nerve physiology and conduction velocities by NAT.

3.1 CONCLUSION

Present animal study revealed that the antioxidant and blood glucose-lowering activity of NAT has a positive effect on the treatment of DN. Overall study has shown that the highest dose that is 400 mg/kg p.o. was found most effective comparatively.

For better results, individual contribution of each phytoconstituents present in *Nyctanthes arbor-tristis* should be studied further with other possible parameters.

3.2 ACKNOWLEDGEMENT

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ABBREVIATIONS

NAT	*Nyctanthes arbor-tristis*
STZ	Streptozotocin
p.o.	Per oral (by mouth)
i.p.	Intraperitoneal
MENA	Methanolic extract of *Nyctanthes arbor-tristis*
DN	Diabetic neuropathy
BSI	Botanical Survey of India
IAEC	Institutional Animal Ethics Committee
CCSEA	Committee for Control and Supervision of Experiment on Animals
GOD-POD	Glucose oxidase-peroxidase
SOD	Superoxide Dismutase
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
EDTA	Ethylenediamine tetraacetic acid
Mn	Manganese
CAT	Catalase
SEM	Standard error mean
ANOVA	Analysis of Variance
AGEs	Advanced Glycation End products
RAGE	Receptor for Advanced Glycation End products
Glib.	Glibenclamide

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