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RESEARCH ARTICLE

# Evaluation of the Neuroprotective Action of *Azadirachta indica* Leaves Extract in Streptozotocin-induced Diabetic Rodent Model

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## Abstract

Among the most common and painful consequences of diabetes mellitus, Diabetic Peripheral Neuropathy (DPN) is one of the most common. For DPN management, a variety of techniques have been used, ranging from traditional medicines to alternative approaches. Natural compounds are also the focus of research to explore the possible treatment by replacing or combining with the existing therapies. Different neurological changes in diabetic neuropathy and the effect of the *Azadirachta indica* (neem) extract were assessed with nerve conduction velocity, and biochemical and histological analysis in Streptozotocin-induced diabetic mellitus. The therapeutic effect of the extract was evaluated with doses 100, 200 and 500mg/kg body weight for 4 weeks after induction of diabetes. The protective effect was evaluated by treating the animals with hydroalcoholic extract of neem leaves in 500mg/kg dose before the induction of diabetes and post-treatment with the standard drug Metformin (500mg/kg). Both resulted in a significant reduction in blood glucose, additionally, 500mg/kg body weight dose revealed the signs of neuroprotection in diabetic rats. Neem leaf extract appears to be promising for future investigations, which might contribute to the emergence of new drugs for diabetes treatment and diabetic neuropathy either alone or in combination with conventional therapies.

**Keywords:** *Azadirachta indica*, Diabetes Mellitus, Hypoglycemic, Neuropathy, Neuroprotective

## 1. Introduction

Diabetes mellitus is a chronic metabolic condition defined by a persistently elevated blood glucose level over a lengthy period (diabetes mellitus). It happens when either the body's insulin production is insufficient or the cells of the body are nonresponsive to the insulin that is generated[1]. Diabetes has risen to the status of a worldwide pandemic illness. Diabetes is expected to affect around 425 million people globally, or 8.8 per cent of individuals aged 20 to 79 years, according to current estimates. Diabetes patients in low- and middle-income

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countries account for around 79 per cent of all diabetes patients[2]. The incidence of type 1 and type 2 diabetes mellitus is growing over the globe, but the prevalence of type 2 diabetes mellitus is increasing considerably more quickly, possibly as a result of increased obesity and a decrease in physical activity as nations become more industrialized. The huge expenditure of the disease is due to its chronicity as well as the associated long-term complications including several macrovascular and microvascular complications. Microvascular problems are substantially more common than macrovascular complications, with the frequency of microvascular complications being much greater[3]. Diabetic Peripheral Neuropathy (DPN) is a frequent consequence of diabetes that affects between 30 and 50 per cent of people with the disease[4]. An internationally acknowledged simplified definition of DPN for clinical practice, according to the American Diabetic Association (ADA), is "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes"[5]. Diabetic peripheral neuropathy leads to sensory loss in the limbs and tingling, burning, deep aching pain may also be apparent. People having DPN may be at an increased risk of developing foot ulcers, which may lead to lower-extremity amputation[6].

To date, there are very few options available for treating diabetic neuropathy. Pregabalin and Duloxetine were already been authorized by the U.S. Food and Drugs Administration (FDA) for pain associated with diabetic neuropathy[7]. Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs), Anti-Epileptic Drugs (AEDs), Tricyclic Antidepressants (TCAs), and capsaicin cream can control the pain only[8]. The therapy of diabetic neuropathy, on the other hand, is not effective in curing the underlying pathogenic process of axonal degeneration or Schwann cell dysfunction in diabetic neuropathy. Furthermore, they are ineffective in a significant number of patients, and there is an urgent need to create effective medicines that block and reverse axonal degeneration that are based on mechanisms of action[9]. Several studies have demonstrated that improvements in glycemic management, blood pressure, and cholesterol levels may lower a person's chance of developing problems[10]. Prevention or delaying the onset of this diabetic complication assumes a greater role in reducing disability and morbidity than non-specific treatment after the occurrence of the complication. Searching for any lead compound that has good hypoglycemic as well as neuroprotective properties for preventing or at least delaying the onset of diabetic neuropathy is important.

Alternative and complementary therapies play an important role in curing patients with diabetic neuropathy symptoms in different ways. Recent research efforts also focus on the discovery of some potential new compounds that could also avoid the frequent side effects associated with conventional therapies[11]. Indian traditional medicine system consists of several medicinal plants, used in various diseases. Previous studies have shown that *A. indica* commonly known as 'neem' in the Indian subcontinent, has antihyperglycemic, hypolipidemic and antioxidant effect[12]. However, there is a lack of studies to explore its neuroprotective role. The purpose of this research was to investigate the neuroprotective effects of the hydroalcoholic extract of *A. indica* leaves in an experimental rodent model.

## 2. Materials and Methods

### 2.1 Animals

Wistar albino rats of 150-200 g weight were used in this study. They were cared for by the criteria of the Committee for Control and Supervision of Experiments on Animals (CPCSEA, 2003). Investigations were carried out with the consent of the R.G. Kar Medical College's Institutional Animal Ethics Committee, in Kolkata, India. All necessary care was taken to avoid unnecessary pain or suffering before, during or after the performance of experiments.

### 2.2 Collection of Plant Material

Leaves of neem (*A. indica*) were procured from the local marketplace and authenticated by the Botanical Survey of India (CNH/2017/Tech.II/43). The leaves were washed extensively to remove any dust, dirt, or other particles that may have stuck to them. A mixer-grinder was used to turn the leaves into powder after they had dried in the shade for fifteen days at room temperature. The coarse powder that has been produced, was then fine-ground even more using a #60 sieve. The resulting fine powder was then maintained

in an airtight container and kept at room temperature, confirming that it was ready for use in future studies.

### 2.3 Preparation of Crude Plant Extract

The plant sample then underwent hot extraction using a continuous Soxhlet extraction apparatus with methanol and water for 48 hours. The extract was collected in a beaker, which was then filtered. After that, the extract was subjected to evaporation at decreased pressure by a rotary evaporator.

### 2.4 Drugs Used

Streptozotocin (STZ) obtained from Sisco Research Laboratories Pvt. Ltd., used for induction of diabetes in experimental animals. Metformin tablets (500 mg) obtained from the market (Glyciphage 500 mg by Franco-Indian Pharmaceuticals Pvt. Ltd.), were used as reference drugs in the study.

### 2.5 Other Chemicals

Glucose, triglyceride, total cholesterol, and HDL estimation kits were obtained from ARKRAY Healthcare Pvt. Ltd., India. HbA1c was estimated using a glycosylated haemoglobin kit obtained from Coral Clinical System, India. Laboratory grade reagent Acetone (≥99.5%) obtained from Sigma-Aldrich, USA.

## 3. Induction and Screening for Diabetes

A single intraperitoneal (i.p.) injection of freshly produced Streptozotocin (STZ) solution 60 mg per kg diluted in 0.1 M cold citrate buffer with pH 4.5 was given to the animals once they had fasted the previous night[13]. 0.5ml solution containing the required STZ dose for each rat injected using a 25G needle. In addition to being provided unrestricted access to food and water, the rats were fed a 15 per cent glucose solution to consume overnight to prevent hypoglycemia. During the third day after the STZ injection, the rats were declared diabetic if their blood glucose levels rose to more than 250 mg/dl. The levels of glucose in the blood were determined utilizing a glucometer (Accusure®) using blood drops obtained from rat tail snapping. The selection of an STZ-induced diabetic rodent model seems justified for evaluating the neuroprotective action of *A. indica* leaves extract due to STZ's ability to induce hyperglycemia and replicate key aspects of diabetic neuropathy. STZ selectively damages pancreatic beta cells, mirroring the pathophysiological conditions of diabetes and associated neurological complications. This model provides a clinically relevant platform to assess the potential neuroprotective effects of *A. indica*, offering insights into its efficacy in mitigating diabetic neuropathic conditions.

### 3.1 Group Divisions of Animals

Animals were divided broadly into two study groups- a) groups to evaluate therapeutic effects and b) groups to evaluate preventive effects. Former groups were subdivided into 6 study groups and later groups were subdivided into 5 study groups. The diabetic control (untreated diabetic) and healthy normal control (non-diabetic non-treated) groups of rats were used for comparison with each group of rats (Table 1).

### 3.2 Parameters Evaluated

Body weights of each animal were evaluated using the digital electrical weighing machine, before induction of diabetes at day 0, and thereafter every 7-day interval up to 28 days. Biochemical parameters like blood glucose estimation were done using Enzymatic GOD-POD, endpoint colorimetry single reagent chemistry method[14]. Pharmacological parameters such as Hot Plate Latency are evaluated by the Hot plate test for hyperalgesia[15] and Paw Withdrawal Frequency is evaluated by the Acetone drop test for cold allodynia[16]. These parameters indicated the sensory function of the nerve. A histopathological study of the sciatic nerve was done. After the research, experimental animals were sacrificed and a right sciatic nerve biopsy was taken. Slides were prepared from the biopsy, thereafter stained with hematoxylin as well as eosin (HE) stain and analyzed under optical microscope (10X and 40X).

### 3.3 Experimental Methodology

All the animals were initially housed for a quarantine period of 7 days. Animals underwent screening tests and suitable animals were properly marked; blood sugar levels and body weights were recorded. The rodents have initially been divided randomly into two broad groups: (i) treatment group and (ii) preventive group. Animals in preventive groups were treated with a preventive dose of 500mg/kg (the most effective

**Table 1.** Distribution of groups to evaluate therapeutic and preventive properties of hydroalcoholic neem extract (*A. indica*) leaves

| Study Group | Description of groups | Total Animal no. (n=78) |
|---|---|---|
| I | Normal control (non-diabetic non treated) (NC) | 6 |
| II | STZ-induced diabetic control (untreated diabetic) (DC) | 6 |
| **Therapeutic effect study** | | |
| III | STZ induced diabetic (D)+ Neem extract in 100 mg/kg (N1) | 6 |
| IV | STZ induced diabetic (D)+ Neem extract in 200mg/kg (N2) | 6 |
| V | STZ induced diabetic (D)+ Neem extract in 500mg/kg (N3) | 6 |
| VI | STZ induced diabetic (D)+ Metformin in 100mg/kg (M1) | 6 |
| VII | STZ induced diabetic (D)+ Metformin in 200mg/kg (M2) | 6 |
| VIII | STZ induced diabetic (D)+ Metformin in 500mg/kg (M3) | 6 |
| **Preventive effect study** | | |
| IX | Pretreatment with Neem extract (N) control animal (N+NC) | 6 |
| X | Pretreatment with Neem extract 500mg/kg (N)+ STZ-induced diabetic (D) | 6 |
| XI | Pretreatment with Neem extract 500 mg/kg (N)+ STZ induced diabetic (D) + Metformin in 100mg/kg (M1) | 6 |
| XII | Pretreatment with Neem extract 500 mg/kg (N)+ STZ induced diabetic (D)+ Metformin in 200mg/kg (M2) | 6 |
| XIII | Pretreatment with Neem extract 500 mg/kg (N)+ STZ induced diabetic (D)+ Metformin in 500mg/kg (M3) | 6 |

dose) for 7 days before the experiment. Thereafter animals in both treatment group and preventive group were subdivided into 11 study groups (6 study groups from treatment group, 5 study groups from preventive group). The diabetic control (untreated diabetic) and healthy normal control (non-diabetic, non-treated) groups of rats were used for comparison with each group of rats. On day 1 of the experiment, all animals were again weighed and blood sugar values were collected. STZ (60mg/kg) injections were prepared and induced in all the animals except group I. On 3$^{rd}$ day the blood sugar values were taken and recorded to establish the development of diabetes. Treatment was initiated for the therapeutic group of animals with different doses of the hydroalcoholic extract of neem and was continued for 4 weeks. Then all therapeutic and preventive groups received Metformin treatment in different doses for 28 days. Then on days 7, 14, 21, and 28, the blood sugar values of all the rats were periodically evaluated. The inclusion of metformin HCl as a comparator in the evaluation of the neuroprotective action of *A. indica* leaves extract in the STZ-induced diabetic rodent model is justified by metformin's established role as a standard oral hypoglycemic agent. Utilizing metformin allows for benchmarking the neuroprotective effects of *A. indica* against a well-documented and widely used therapeutic intervention in diabetes, providing a comparative reference for assessing the potential benefits of the plant extract in the context of diabetic neuropathy. Finally, all blood sugar values except day 28 values were measured by using a glucometer (Accusure®) using blood drops obtained from rat tail snapping. On day 28, blood was obtained from tail veins and tests for blood parameters were estimated along with body weight, hot plate latency, and paw withdrawal frequency by acetone drop test. Rats were euthanized and sciatic nerve biopsies were taken.

## 3.4 Statistical Analysis

Statistical analysis was done with the help of MS EXCEL, GRAPHPAD PRISM v7.0 (GraphPad Software, San Diego, CA, USA; www.graphpad.com). All of the variables were computed as mean ± SEM. To do a statistical comparison between diabetics and other

groups, the ANOVA test was employed, followed by the Dunnet Multiple comparison test. The significance threshold was set at $p<0.05$.

## 4. Results and Analysis

The current investigation was conducted on 78 Wistar rats weighing between 150-200 grams in the Pharmacology Department at R.G. Kar Medical College, Kolkata, India, during the study period from February 2017- September 2018.

### 4.1 Estimation of Bodyweight

Initial and 28$^{th}$-day body weight measurements were taken on all rats in each group. It was noticed that in comparison to the diabetic control Group (II), there was a substantial rise in body weight in neem extract-treated groups at 200mg/kg and 500mg/kg doses, comparable with the Metformin-treated groups (Table 2). The pretreated groups with neem extract (500mg/kg dose) alone and also the Group XI, XII and XIII which were pretreated with neem extract (500mg/kg dose) as well as post-diabetes treatment was done with Metformin in different doses revealed significant percentage changes in body weight compared to the diabetic control rats. Among those groups, Group VIII (Metformin 500mg/kg treated diabetic rat) and Group V (neem 500mg/kg treated diabetic rat) showed a maximum increase in body weight with 7.15±2.04% and 6.78±0.55% respectively.

### 4.2 Estimation of Blood Sugar

On day 28, the diabetic control group (group II) was showing the highest blood sugar as no therapy was provided to this group. Group XIII (diabetic rats pretreated with neem extract 500mg/kg and post-diabetes treatment with Metformin 500mg/kg orally) showed the lowest blood sugar value among all the treated groups. A substantial drop in blood sugar level was observed in the pretreated groups on day 28. Table 3 revealed that statistically significant ($p<0.05$) percentage changes in blood sugar levels to control, were noticed in all the groups treated with neem extract, which were equivalent to that of the standard drug-treated groups. Group VIII (diabetic rats treated with metformin 500 mg per kg orally) showed the highest reduction in blood sugar level between day 3 and day 28 (50.15±0.1366%).

**Table 2.** Distribution of percentage changes in blood sugar between day 3 and day 28 among different experimental groups (n=78)

| Group | Description of groups | Blood sugar day 3 (mg per dl) | Blood sugar day 28 (mg per dl) | % changes in blood sugar between day 3 and day 28 |
|---|---|---|---|---|
| I | NC | 88.33±2.23 | 90.82±3.27 | 3.08±4.171 |
| II | DC | 344.33±7.39 | 342.83±2.12 | -0.1416±2.307 |
| III | D+N1 | 361.17±1.85 | 324.17±1.99 | -10.18±0.4649* |
| IV | D+N2 | 369.83±4.07 | 313.98±2.00 | -15.04±1.249* |
| V | D+N3 | 343.83±2.57 | 192.67±2.44 | -43.89±0.3445* |
| VI | D+M1 | 370.17±4.62 | 307.05±1.65 | -17.01±0.6133* |
| VII | D+M2 | 350.33±3.80 | 242.88±2.50 | -30.67±0.151* |
| VIII | D+M3 | 361.33±3.25 | 180.13±1.56 | -50.15±0.1366* |
| IX | N+NC | 85.17±3.07 | 81.53±1.9 | -3.873±2.9 |
| X | N+D | 298.5±6.99 | 215.898±2.64 | -27.44±2.156* |
| XI | N+D+M1 | 294.83±5.05 | 192.11±6.73 | -34.8±2.196* |
| XII | N+D+M2 | 299.67±3.57 | 181.10±6.74 | -39.57±2.126* |
| XIII | N+D+M3 | 280.17±5.60 | 149.74±5.6 | -46.5±2.053* |

$p<0.05$, **$p<0.01$,***$p<0.001$ One-way ANOVA followed by Dunnet's multiple comparison tests was used to compare % changes in the blood glucose level between the diabetic control group with other groups. Mean ± SEM. In percentage change, the + symbol corresponds to the rise in body weight and the – symbol denotes a reduction in body weight to the control group of rats.

### 4.3 Estimation of Pharmacological Parameter

Two pharmacological parameters were evaluated in the study. Hot plate latency period and paw withdrawal frequency by acetone drop test were measured on day 28 for detecting the hyperalgesia and cold allodynia respectively, associated with diabetic neuropathy.

### 4.4 Hot Plate Test

The hot plate test was done on day 28 of the experiment. Table 4 indicated that hot plate latency in Group II (diabetic control) was significantly low compared to both the normal control group (Group I) and the normal control group pretreated with neem extract (Group IX) at day 28. This indicated that hyperalgesia was maximum in diabetic control and minimum in

**Table 3.** Distribution of hot plate latency period for individual groups at day 28 (n=78)

| Group | Description of groups | Hot plate latency (sec) | p-value |
|---|---|---|---|
| I | NC | 8.167±0.79** | <0.001 |
| II | DC | 2.167±0.48 | - |
| III | D+N1 | 3.167±0.30 | 0.997 |
| IV | D+N2 | 4.667±0.56 | 0.476 |
| V | D+N3 | 7.167±0.98** | 0.009 |
| VI | D+M1 | 3.167±0.40 | 0.997 |
| VII | D+M2 | 4.833±0.48 | 0.399 |
| VIII | D+M3 | 7.333±0.76** | 0.006 |
| IX | N+NC | 8.833±0.91*** | <0.001 |
| X | N+D | 3.667±0.33 | 0.934 |
| XI | N+D+M1 | 5.833±0.87 | 0.103 |
| XII | N+D+M2 | 6.833±2.01* | 0.018 |
| XIII | N+D+M3 | 7.167±2.14** | 0.009 |

p<0.05, **p<0.01,***p<0.001 One-way ANOVA followed by Dunnet's multiple comparison test was used to compare the changes in the latency period between the diabetic control group with other groups. Mean ± SEM.

**Table 4.** Distribution of percentage of Paw Withdrawal Frequency (PWF%) (n=78)

| Group | Description of groups | PWF (%) | p-value |
|---|---|---|---|
| I | NC | 16.67±3.33 *** | <0.001 |
| II | DC | 58.33±3.07 | - |
| III | D+N1 | 43.33±5.58 | 0.335 |
| IV | D+N2 | 40±3.65 | 0.145 |
| V | D+N3 | 15±3.42*** | <0.001 |
| VI | D+M1 | 41.67±5.43 | 0.225 |
| VII | D+M2 | 38.33±6.01 | 0.089 |
| VIII | D+M3 | 20±3.65*** | <0.001 |
| IX | N+NC | 15±3.42*** | <0.001 |
| X | N+D | 36.67±9.19 | 0.053 |
| XI | N+D+M1 | 26.67±9.19** | 0.001 |
| XII | N+D+M2 | 21.67±4.77*** | <0.001 |
| XIII | N+D+M3 | 18.33±4.77*** | <0.001 |

p<0.05, **p<0.01,***p<0.001 One-way ANOVA followed by Dunnet's multiple comparison test was used to compare % changes in the paw withdrawal frequency between the diabetic control group with other groups. Mean ± SEM.

healthy normal control groups as the nerves were intact in those two non-diabetic groups. While exploring the therapeutic effect among the treated groups, Group V (diabetic rats treated with neem extract 500mg/kg) and Group VIII (diabetic rats treated with Metformin 500 mg kg) showed significant ($p<0.01$) increased hot plate latency compared to the diabetic control group. Groups XII and XIII, where the rats were pretreated with neem extract (500 mg per kg) and after diabetes induction treated with Metformin 200mg/kg and 500mg/kg respectively also revealed significantly high values of hot plate latency. These indicated that neem and metformin can decrease hyperalgesia.

## 4.5 Acetone Drop Test

All the experimental animals of each group were tested for paw withdrawal frequency by acetone drop test at day 28 and were compared with the diabetic control to measure the percentage paw withdrawal frequency (% PWF). Groups with a high value of this paw withdrawal frequency had increased cold allodynia and vice versa. It was seen from Table 4 that Group II (diabetic control group) had the highest paw withdrawal frequency (%) with 58.33±3.07 sec. The lowest paw withdrawal frequency was seen in Group V (neem 500mg/kg treated diabetic rat) among all the treated groups with 15±3.42 sec. Among the other diabetic groups for exploring the therapeutic effect of neem leaf extract, Group VIII (diabetic rats treated with Metformin 500 mg per kg) had significantly low paw withdrawal frequency. On the other hand, the diabetic groups evaluating the preventive role of neem leaf extract, Group XI ($p<0.01$), Group XII ($p<0.001$) and Group XIII ($p<0.001$) showed statistically significant low paw withdrawal frequency compared to the diabetic control. These results showed both neem and Metformin were able to decrease cold allodynia than the diabetic control rats.

## 4.6 Histological Study of Sciatic Nerves of Different Groups

Sciatic nerves were dissected from the right legs of the experimental rats after sacrificing those following CPCSEA norms. Nerve tissues were fixed with 10% formalin-containing vials and histological studies were performed using hematoxylin and eosin (H and E) stain to observe the therapeutic and preventive effect of neem leaf extract on diabetic neuropathy. Figure 1 showed that normal rats and neem-pretreated normal rats retained normal architecture of the sciatic nerve. STZ diabetic control and neem pretreated diabetic control group showed severe oedema (e), degenerative (arrow) changes, and decreased nuclear materials. Those changes were less evident in the slides obtained from Groups V, and VIII, i.e., the diabetic rats treated

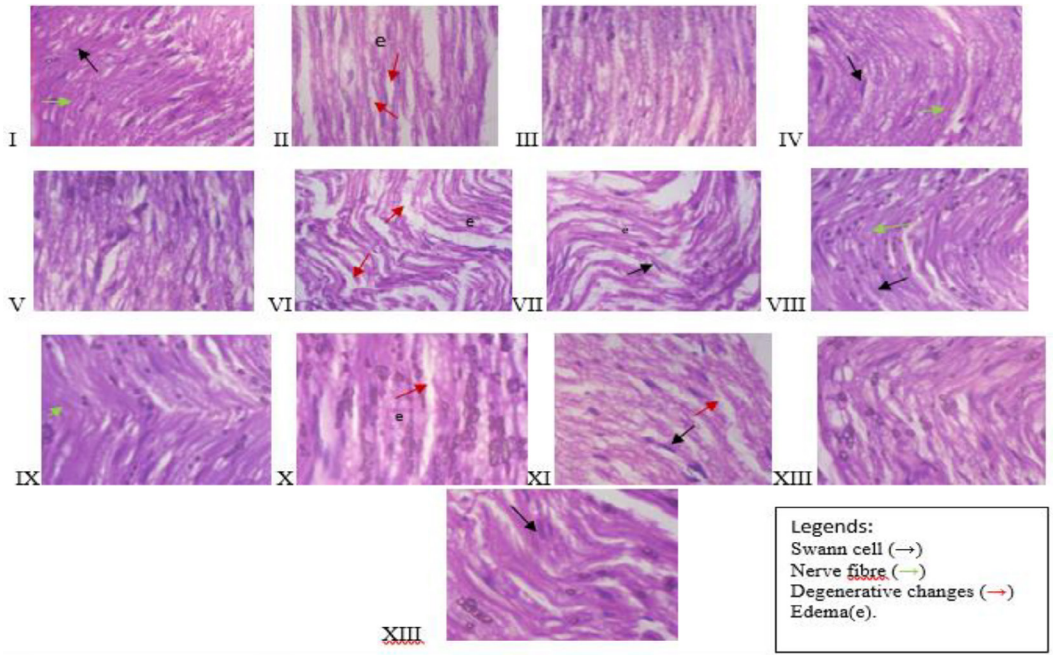


**Figure 1.** Histological evaluation of sciatic nerve of different groups (HE stain in 40X).

with neem 500 mg per kg and Metformin 500 mg per kg respectively. The normal nerve structure preservation was also significant in Group XIII where the rats were pretreated with neem extract (500mg/kg) and after diabetes induction treated with Metformin 200mg/kg.

## 5. Discussion

According to the findings of the current research, there was a significant weight reduction in the diabetic control group after day 28. This might be because metabolic demand in diabetic situations is met by the breakdown of stored carbohydrates and fats. The breakdown of fat in the adipose tissue and the stored glycogen of the body (liver/muscular tissue) trigger the wasting of muscles and decrease in the overall body weight[17]. The reduction of body weight might be due to dehydration associated with diabetes. A study conducted in Iran by Akbarzadeh *et al*., 2007 showed weight reduction in the diabetic control group which was in agreement with the present study[18]. It also showed that both hydroalcoholic NLE (200mg/kg and 500mg/kg) and metformin (200mg/kg and 500mg/kg) treatment up to day 28 significantly increased the body weight of the diabetic rats with $p<0.001$. Interestingly, this discovery is consistent with the findings of research done at the Bangladesh Agricultural University in Bangladesh in 2010[19].

Neem Leaf Extract (NLE) as well as Metformin treatment showed a reduction in blood glucose levels at the end of the study without any instances of fatality. The percentage reduction was 43.89% and 50.15% respectively for the neem extract (500mg/kg) and Metformin (500mg/kg) treated groups respectively. This was in agreement with the study conducted by Das *et al*. in 2010, where 17.89% and 32.10 % reductions were shown with Metformin and Neem (both given 500mg/kg p.o.) treated group respectively in 21 days[19]. However, in contrast to another study conducted by Chang *et al*., 2005, Neem leaf extract in 250mg/kg was reported to be the more potent dose in the reduction of blood sugar level[20]. NLE pretreated diabetic group (group X) showed significantly less value on day 3 and day 28 (298.5±6.99 mg/dl and 215.898±2.64 mg/dl, Mean ± SEM) compared to the diabetic control group. The NLE pretreated diabetic rats treated with Metformin groups also showed significantly low day 28 blood sugar values. This outcome signified that neem has a preventive role before the induction of diabetes by reducing the severity of onset as well as increasing the responsiveness to further antidiabetic treatment. These findings were in agreement with the study conducted on rabbits by Khosla *et al*.[21].

Metformin reduces blood sugar by activating hepatic AMP-activated protein kinase (AMPK) which is an indirect consequence of interference with cellular respiration and lowering of intracellular ATP and other energy sources[22]. It has been proposed by Sharma *et al*.,[23] that the hypoglycaemic impact of neem is due to extra-pancreatic sites of action, such as enhanced peripheral glucose consumption, or that it is due to a direct metabolic action on tissues, notably the liver, similar to that of metformin. Thereby help to overcome insulin resistance. Metformin also does not cause hypoglycemia in normoglycemic patients. However, there may be the possibility of neem having a hypoglycemic effect similar to insulin as suggested by previous studies[16].

Assessment of pharmacological parameters to evaluate the neuroprotective role of NLE in diabetic neuropathy in diabetic control rats had similar findings as that of Fox *et al.,* and Yoon *et al.,* indicating the development of hyperalgesia and cold allodynia in the diabetic control groups[15,16]. Neem 500mg/kg treated diabetic group (15±3.42 sec, Mean±SEM) and Metformin 500mg/kg treated diabetic group (20±3.65 sec, Mean±SEM) showed significant improvement of hyperalgesia by lowering hot plate latency. Those two groups also showed improvement in cold allodynia by decreasing paw withdrawal frequency significantly compared to the diabetic group. In preventive groups (500mg/kg neem pretreated diabetic rats treated with metformin 200mg/kg and 500mg/kg) dose-dependent improvement of those two pharmacological parameters was also noted. In one investigation performed by Morani *et al*.[24] where alloxan-induced diabetic rats were treated with hypoglycemic drug pioglitazone. It showed an increased hot plate latency period compared to the diabetic untreated group. Researchers opined that strict glycemic control has a strong positive correlation with neuroprotection. Pioglitazone and metformin both act in diabetes by sensitizing tissues to insulin overcoming insulin resistance. Both are very effective

in maintaining glycemic control. That could explain the present study's finding of the effects of metformin on the aforementioned pharmacological parameters related to neuropathy. Neem also has been shown to produce a strict glycaemic control similar to metformin in the present study.

Another study done in Iran by Farshid *et al.*,[25] showed that paw withdrawal frequency decreased in STZ diabetic rats treated with insulin. They interpreted the observation as an improvement in cold allodynia in diabetic neuropathy and deduced that insulin improved by producing strict glycaemic control. In the present study also neem and metformin produced strict glycaemic control. Hence reduction of cold allodynia is by that study. In the case of groups to evaluate the preventive effect of NLE on diabetic neuropathy, pretreatment with neem was shown in the present study to achieve strict blood sugar control compared to the groups to evaluate the therapeutic effect of NLE. This could be the cause of its effectiveness in improving the hyperalgesia and cold allodynia which is by the previous findings[26].

Sciatic nerve tissue changes, including fibre degeneration and oedema, were detected in the current analysis, which is consistent with the findings of Omran *et al.*,[27], who found that STZ-induced diabetic peripheral neuropathy in rats resulted in partial separation of myelinated nerve fibres, endoneurial oedema, axonal atrophy, and fibre degeneration of the sciatic nerve. NLE and metformin in both groups to evaluate the therapeutic effect and preventive effect of neem in diabetic neuropathy had shown decreased histopathological changes in sciatic nerve biopsy which were probably due to its anti-hyperglycemic, anti-hyperlipidemic effects. Better controlling of blood sugar reduces oxidative stress as well as neurodegeneration in diabetic rats. This fact is well supported by Farshid *et al.*,[25] in their study where they opined neuroprotective effect of insulin due to reduction in blood sugar and subsequent oxidative stress. The present study revealed some important directions for some therapeutic approaches for the prevalent chronic disease of diabetes. Detailed animal experiments in other different models may be conducted to explore a mechanical aspect of the disease as well as the modalities of the treatment. Further confirmation can be established by clinical research.

## 6. Conclusion

The present study evaluated the role of *A. indica* in reducing blood sugar levels in diabetes. Delayed progression of hyperalgesia, and cold allodynia after treatment with neem leaf extract might be an indication of the protective action against the progression of diabetic neuropathy. Thus *A. indica* leaf extract appears to be promising for future research, with the potential for medication development for diabetes and diabetic neuropathy. Further exploration of the exact mechanism of the hydroalcoholic extract of neem leaves in producing glycemic control and neuroprotective action needs to be established.

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