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Effect of *Solanum torvum* Swartz on diabetic neuropathy in alloxan-induced diabetic rats

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Solanum torvum Swartz is a well-known traditional herbal medicinal plant used in diabetes and diabetes-related complications. The objective of the study was to evaluate the effect of *S. torvum* on diabetic neuropathy in alloxan-induced diabetic rats. Diabetes was induced in Wistar rats by using a single intraperitoneal injection of alloxan monohydrate (150 mg/kg; i.p.). After confirmation of diabetes, rats received metformin (120 mg/kg, p.o.) and STME (30 and 100 mg/kg, p.o) for 5 weeks. Diabetic rats showed significant (P <0.05) behavioural changes, increase in blood glucose levels, decrease in relative organ weight of pancreas, significant (P <0.05) decrease in reduced glutathione (RGSH) and significant (P <0.05) increase in TBARS levels. While STME (100 mg/kg) treated diabetic rats significantly (P <0.05) reversed the above parameters as compared to diabetic rats. Treatment with STME (100 mg/kg) has also reversed histopathological changes as observed in diabetic control rats. The study suggests that methanolic extract of *S. torvum* ameliorates diabetic neuropathy in alloxan-induced diabetic rats.

Keywords: Alloxan monohydrate, Diabetic neuropathy, Metformin, *Solanum torvum.*

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/81, A61K 131/00, A61P 3/00

Introduction

Diabetic neuropathy (DN) is a chronic complication of both type I and type II diabetes mellitus (DM). Patients with long term diabetes may develop complications affecting the eyes, kidneys or nerves (microvascular complications) or major arteries. Diabetic neuropathy is nerve damaging disorder associated with DM. Neuropathic pain is characterized by the sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to the stimulus that does not normally provoke pain (allodynia). Various proposed mechanisms which lead to pathogenesis of DN are activated polyol pathway, AGE's (Advanced glycation end products) formation PKC (Protein kinase C) activation and hexosamine pathway. Hyperglycemia is the primary cause of DN. There has been a major advance in the control of hyperglycemia (diabetes), through dietary changes, hypoglycemic agents, insulin and islet transplantation, even though the long term complication of diabetes, such as neuropathy remains a serious problem[1]. Therefore, agents or compounds that exert multiple actions, such as antioxidants, antidiabetic/hypoglycemic and antiglycation properties could be more effective than agents with a single action[2]. Alloxan-induced diabetes is one of the commonly used models to induce DM in the experimental animal. Alloxan has found to be selectively toxic to pancreatic beta cell as it preferentially accumulates in the beta cells as glucose analogues. In addition, the cytotoxic action of alloxan is mediated mainly by the generation of reactive oxygen species (ROS). Alloxan and its reductive product, dialuric acid, has been noted to develop redox cycle with the formation of superoxide radicals, which undergo dismutation to hydrogen peroxide [3]. Alloxan is the most leading chemical compound used in diabetogenic research. In research, it is used for induction of Type 1 diabetes. Alloxan is urea derivative which causes selective necrosis of β-cells of pancreatic islet. Chemical induction with alloxan appears to be the easiest, reliable and the most practicable method of inducing diabetes mellitus in rodents[4]. It is generally used to induce experimental diabetes in an animal such as rabbits, rats, mice and dogs with different grades of disease severity by varying the dose of alloxan used[5].

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Solanum torvum Swartz (Family Solanaceae) is commonly known as Turkey berry. It possesses antimicrobial[6], antiviral[7], immunosecretory[8], antiulcer[9], antioxidant[10], analgesic and anti-inflammatory[11] activities in animal models. In earlier studies, cardioprotective[12], hepatoprotective[13], and nephroprotective[14] activity of *S. torvum* against doxorubicin-induced toxicities in Wistar rats have been studied, and antihypertensive and metabolic correction activity in fructose hypertensive rats[15] has also been reported. The plant due to their immunomodulatory and antioxidant property may be used in the treatment of benign prostatic hyperplasia[16]. The alkaloid fraction of leaf extract of *S. torvum* has been reported to be neuroprotective on *Drosophila melanogaster* against β-Amyloid induced Alzheimer disease[17]. Dietary flavonoids and alkaloids exert cardioprotective, chemopreventive, and neuroprotective effects too. The biological activities of phytoconstituents have been attributed to their antioxidant, antiinflammatory, and signalling properties[18]. Some plants of genus *Solanum* have been used in folk medicine as antidiabetic[19,20]. In view of the above literature, the present study has been designed to evaluate the ameliorative effect of *S. torvum* against diabetic neuropathy in alloxan-induced diabetic rats.

Materials and Methods

Drugs and chemicals

Alloxan monohydrate (Sigma-Aldrich Chemicals Pvt. Ltd., USA.), Ascorbic acid (Research lab), Pet ether 60-80 ^{o}C (Research lab), Methanol (Research lab), Metronidazole (Kirti Pharma, Musalgaon, Nasik), Acetone (Sigma), TBA (Sigma) & TCA (Research lab), DTNB (Sigma) and glucometer (Accucheck) were used for the study.

Preparation of extract

Seeds of *S. torvum* were purchased from Tirunelvelli district of Tamil Nadu in May 2017 and authenticated from Ayurved Sanshodhan Vibhag, Nashik (Voucher specimen No-ASV0978). Mature fruits were dried in shade and grounded. The powder obtained was defatted using pet ether (60–80 ^{o}C). The marc obtained was dried and further extracted with methanol solution using soxhlet extractor. The extract obtained was dried under the vaccum in reduced pressure (1400 psi) then evaporated to obtain 11.6% w/w of methanolic extract of *S. torvum*. The pH of the methanolic extract was found to be 6.8[21].

Animals

Wistar rats of either sex weighing around 150-200 g were purchased from Bombay Veterinary College, Parel. Animals were housed separately in groups of 6 animals per cage under standard laboratory conditions maintained at a temperature (22-24 °C) and humidity (50-60%) with light and dark cycle of 12 hours each. The animals were acclimatized for at least five days before behavioural studies. All experiments were carried out during day time between 09:00 and 16:00 hours. All animals had proper access to standard food and water. Study protocol was approved by the Institutional Animal Ethics Committee (MGV/PC/CPCSEA /XXXIV/01/2017/02), Government of India, New Delhi.

Experimental design

Animals were divided into 5 groups of 6 rats (either sex) each and treated for 5 weeks. Group I Normal control received vehicle as saline only (10 mL/kg). Group II Rats treated with Alloxan monohydrate (150 mg/kg; i.p.) once only. Group III Diabetic rats treated with metformin (120 mg/kg; p.o.) once every day for 5 weeks. Group IV diabetic rats treated with *S. torvum* methanolic extract (30 mg/kg; p.o.) once every day for 5 weeks. Group V diabetic rats treated with *S. torvum* methanolic extract (100 mg/kg; p.o.) once every day for 5 weeks. During the treatment schedule, all groups were subjected to morphological and behavioural tests once a week for 5 weeks. At the end of the treatment schedule, antioxidant, relative organ weight and histopathological tests were performed.

In vitro test

In vitro glucose uptake by yeast cell

The commercial baker's yeast in distilled water was subjected to centrifugation (3000× g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of suspension was prepared in distilled water. Various concentrations of plant extracts (5-100 µg/mL) were added to 1 mL of glucose solutions (5, 10, and 25 mM) and incubated together for 10 minutes at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by incubation at 37 °C for 60 minutes. After 60 minutes, the tubes were centrifuged at 2500 rpm for 5 minutes (R-24) and amount of glucose was estimated in the supernatant[21]. Metronidazole was used as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Increase in glucose uptake (%) = Abs (sample) – Abs (control) /Abs (sample) ×100

Morphological study

Body weight and water intake were monitored once in a week.

Behavioural study

Tail immersion test (Cold water)

Distal 5 cm of rat's tail was immersed in cold water maintained at 10 °C. Time taken for withdrawal of tail from cold water was noted. A cut off time of 20 seconds was maintained to prevent tissue injury. The procedure was repeated 3 times for each animal. The decrease in tail contact time with cold water, indicated nociception, whereas prolonged contact time indicated anti-allodynic effect[22].

Tail immersion test (Hot water)

Heat hyperalgesia was measured by immersion of the terminal part of the tail (1 cm) in warm water maintained at 52.5±0.5 °C. The duration of tail withdrawal reflex was recorded as a response of heat thermal sensation and a cut off time of 15 seconds was maintained. Shortening of tail withdrawal time is an indicator of thermal hyperalgesia[23].

Acetone drop method

Cold chemical thermal sensitivity was assessed using acetone drop method. Rats were placed in a metal mesh cage and allowed to habituate for approximately 20 minutes. Acetone drop (50 µL) was applied gently on to the mid plantar surface of the hind paw. It generates a cold chemical sensitive reaction that is paw liking, shaking or rubbing the hind paw with brisk foot withdrawal after application (2-5 sec) of acetone which was considered as anti-nociceptive effect[24].

Rota rod test

This test was conducted using rota rod apparatus by placing rats on 15 rpm rotating spindle. The fall off time of each rat from rotating spindle was recorded during 5 minutes period[25].

Hot plate method

The nociceptive threshold for heat is an index for thermal hyperalgesia. The plate was preheated and maintained at a temperature of 52.5±2 °C. A rat was placed on the hot plate and nociceptive threshold with respect to licking of the hind paw or jumping will be recorded in seconds. The onset of licking and jumping response was recorded. The cut off time of 20 seconds was maintained[26].

Blood glucose levels

Rats were kept in restrainers; their tail was cleaned with warm soap solution using cotton. Blood samples were withdrawn from the tail vein under mild isoflurane anaesthesia. Blood glucose levels were monitored after 72 hours of induction of diabetes by alloxan monohydrate (150 mg/kg, i.p.). Blood glucose levels were monitored by using a glucometer (Accucheck) to confirm hyperglycemia. Hyperglycemic rats (glucose level >200 mg/dL) were separated and selected for the study. Blood glucose levels were also measured at the end of 5 weeks of treatment in all groups.

Antioxidant study

Preparation of serum and tissue homogenate

Under light isoflurane anaesthesia blood samples were withdrawn by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 10 minutes using REMI-Centrifuge (R-24). The serum samples were maintained at (-20 °C) to be used for measurement of RGSH. The pancreas was dissected out, immediately washed in ice-cold saline, dried and weighed for measurement of relative organ weight and homogenized (REMI motors, RQ-127A) in ice-cold 0.1 M Tris-HCl buffer for estimation of TBARS.

RGSH (Reduced Glutathione)

About 1 mL homogenate (pancreatic) was added to 1 mL of 10% trichloroacetic acid, followed by centrifugation. Then, 1 mL supernatant was collected and 5 mL Ellman's reagent in 100 mL of 1% sodium citrate and 3 mL of phosphate buffer (pH 8) was added. Change in colour was noted. Absorbance was taken at 412 nm and results were expressed as nM/mg of wet tissue of RGSH activity[27].

TBARS (Thiobarbituric Acid)

Test tubes containing 100 µL serum, 1 mL trichloroacetic acid (20%) and 1 mL thiobarbituric acid reagent (1%) were incubated at 100 °C for 30 min, followed by cooling on ice bath and centrifugation at 1000 g (20 minutes). Absorbance was taken at 532 nm[28].

Histopathological analysis of sciatic nerve

Histopathological study was done at Histopathological lab (Clinpath lab, Nashik). The left thigh area was shaved and then prepared for surgery. The sciatic nerve was exposed through a skin incision and gluteal muscle splitting. Then sciatic nerve was

cut into 4 μmm thickness and then kept in the 10% formalin (fixation solution). Staining was done using hematoxyline and eosin. Then cross-sections were observed using a light microscope (100×) for axonal degeneration and vascular defects[29].

Statistical analysis

Data were analyzed using PRIMER statistical software and expressed as Mean±SEM. Statistical analysis was done using One-way ANOVA, followed by Dunnett's test. **P* value <0.05 was considered statistically significant.

Results

***In vitro* glucose uptake by yeast cell**

Percentage uptake of glucose by yeast cells was increased with increasing dose of STME at various concentrations of glucose (5, 10, and 25 mM). The methanolic extract of *S. torvum* at higher concentrations (above 40 μg/mL) exhibited glucose uptake activity comparable with standard metronidazole solution (Fig. 1-3).

Morphological tests

Body Weight

After second week of induction of diabetes, there was significant (*P* <0.05) decrease in body weight of diabetic control rats as compared to normal control rats. Diabetic rats treated with standard metformin (120 mg/kg) and diabetic rats treated with *S. torvum* methanolic extract (30 and 100 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 4th and 5th week of treatment schedule (Fig. 4).

Water intake

There was a decrease in water intake in diabetic control rats as compared with normal control, metformin and STME treated diabetic rats.

Behavioural tests

Tail immersion in cold water

Cold allodynia was observed in the diabetic rats at the 3rd week of induction of diabetes. Cold allodynia was indicated by shortening of tail withdrawal latency as compared to normal rats. Diabetic rats treated with metformin (120 mg/kg) and *S. torvum* methanolic extract (100 mg/kg) showed significant (*P* <0.05) improvement in tail withdrawal latency in 3rd, 4th, and 5th week of treatment schedule as compared to diabetic control rats (Fig. 5).

Tail immersion in hot water

Heat hyperalgesia was observed in diabetic rats at the 3rd week of induction of diabetes. Heat

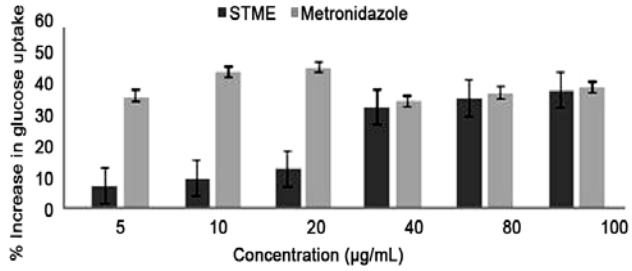

Fig. 1 — The comparative % increase in glucose uptake by yeast cells by *Solanum torvum* and Standard drug Metronidazole at 5 mM glucose concentration.
(Values are expressed as mean±SD, N=6) ; STME- *Solanum torvum* methanolic extract.

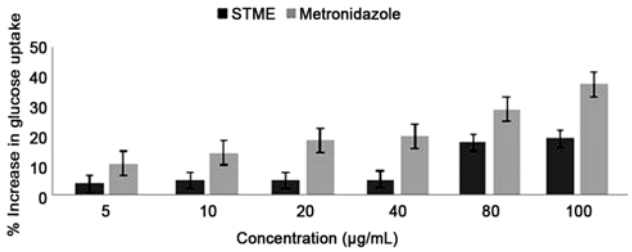

Fig. 2 — The comparative % increase in glucose uptake by yeast cells by *Solanum torvum* extract and Standard drug Metronidazole at 10 mM glucose concentration.
(Values are expressed as mean±SD, N=6); STME- *Solanum torvum* methanolic extract.

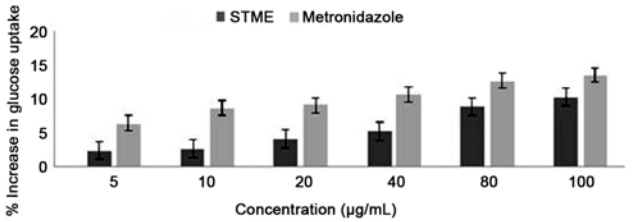

Fig. 3 — The comparative % increase in glucose uptake by yeast cells by *Solanum torvum* and Standard drug Metronidazole at 25 mM glucose concentration.
(Values are expressed as mean±SD, N=6); STME- *Solanum torvum* methanolic extract.

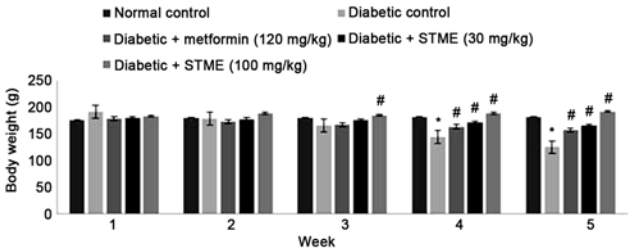

Fig. 4 — Effect of STME (30 and 100 mg/kg) and Metformin (120 mg/kg) on body weight in Alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. **P* <0.05 as compared to normal control group and # *P* <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

hyperalgesia was indicated by a significant reduction in tail withdrawal latency as compared to normal rats. Diabetic rats treated with metformin (120 mg/kg) and *S. torvum* methanolic extract (100 mg/kg) showed significant (P <0.05) improvement in tail withdrawal latency in 3rd, 4th, and 5th week of treatment schedule as compared to diabetic control rats (Fig. 6).

Acetone drop method

In this test, acetone drop applied to the plantar surface of the hind paw of diabetic rats resulted in cold allodynia, it was indicated by a decrease in reaction time as compared to normal rats. Animals treated with standard metformin (120 mg/kg) showed significant (P <0.05) decrease in reaction time at 3rd , 4th and 5th of treatment schedule as compared to diabetic control rats. While animals treated with *S. torvum* methanolic extract (100 mg/kg) showed significant (P <0.05) decrease in reaction time at 3rd and 4th week of treatment schedule as compared to diabetic control rats (Fig. 7).

Rota rod test

Diabetic rats showed motor incoordination as indicated by significant (P <0.05) decrease in fall off time using rota rod apparatus. Diabetic rats treated with metformin (120 mg/kg) and *S. torvum* methanolic extract (30 and 100 mg/kg) showed significant (P <0.05) improvement in motor coordination as indicated by a decrease in fall off time as compared to diabetic control rats after 2nd week of treatment schedule as compared to diabetic control rats (Fig. 8).

Hot plate method

Heat hyperalgesia in diabetic rats was observed at the 3rd week of induction of diabetes. Heat hyperalgesia was indicated by a significant reduction in paw withdrawal latency as compared to normal rats. Diabetic rats treated with metformin (120 mg/kg) and *S. torvum* methanolic extract (100 mg/kg) showed significant (P <0.05) improvement in paw withdrawal latency at 3rd, 4th, and 5th week of treatment schedule as compared to diabetic control rats (Fig. 9).

Blood glucose levels

After treatment, blood glucose levels significantly (P <0.05) decrease in diabetic rats treated with metformin (120 mg/kg) and diabetic rats treated with *S. torvum* methanolic extract (100 mg/kg) as compared to diabetic control rats (Fig. 10).

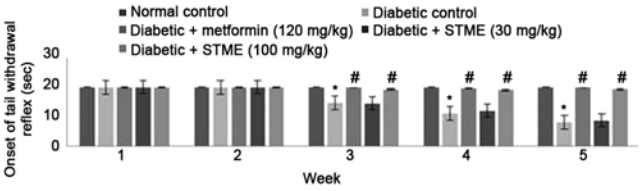

Fig. 5 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on cold allodynia assessed by tail immersion in cold water test in alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compare to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

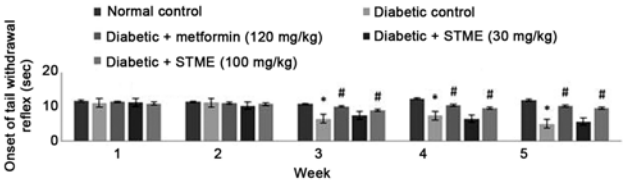

Fig. 6 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on heat hyperalgesia assessed by tail immersion in hot water test in alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

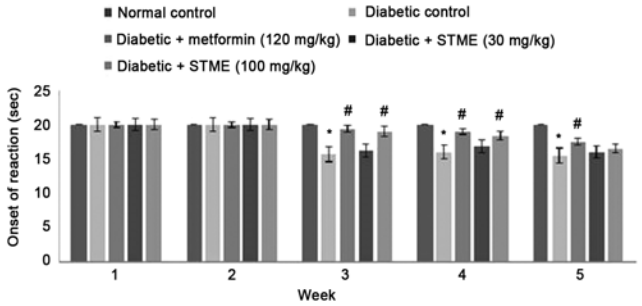

Fig. 7 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on cold allodynia assessed by the acetone drop test in alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

Relative organ weight

There was significant (P <0.05) decrease in relative organ weight of pancreas of animals of diabetic control rats as compared with normal control rats, and significant (P <0.05) increase in relative organ weight of pancreas in diabetic rats treated with standard metformin (120 mg/kg) and in diabetic rats treated with *S. torvum* methanolic extract (30 and 100 mg/kg) as compared to diabetic control rats (Fig. 11).

Antioxidant studies

In-vivo antioxidant studies like RGSH, TBARS levels were estimated by performing various standard procedures.

Reduced glutathione

RGSH is a primary antioxidant in the cell. significant (P <0.05) decrease in the amount of RGSH was observed in the diabetic control group compared with the normal control group, while in diabetic rats treated with metformin and *S. torvum* methanolic extract (30 and 100 mg/kg) showed a significant increase in RGSH levels as compared with the diabetic control group (Fig. 12).

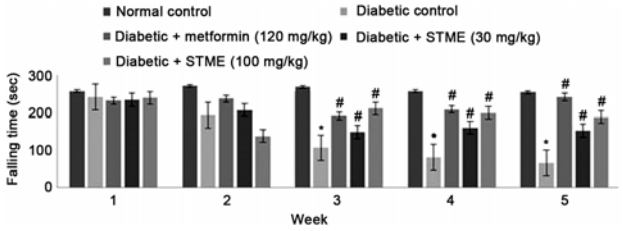

Fig. 8 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on motor coordination assessed by the rota rod test in alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compare to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

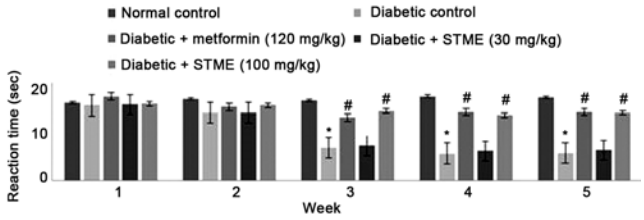

Fig. 9 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on heat hyperalgesia assessed by hot plate method in alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

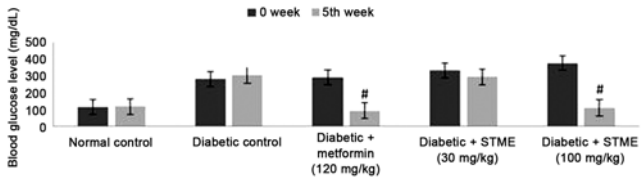

Fig. 10 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on blood glucose levels of alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

Thiobarbituric acid

There was significant (P <0.05) increase in TBARS levels in the diabetic control rats as compared to normal rats, while diabetic rats treated with metformin, *S. torvum* methanolic extract (30 and 100 mg/kg) showed significant (P <0.05) decrease in TBARS levels as compared with diabetic control rats (Fig. 13).

Histopathological examination of the sciatic nerve

The sciatic nerve of diabetic rats developed severe pathological changes as compared with the groups

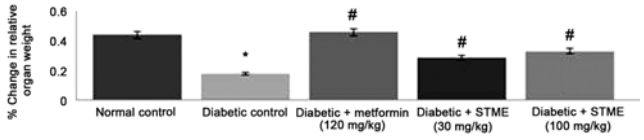

Fig. 11 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg)on relative organ weight of alloxan induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

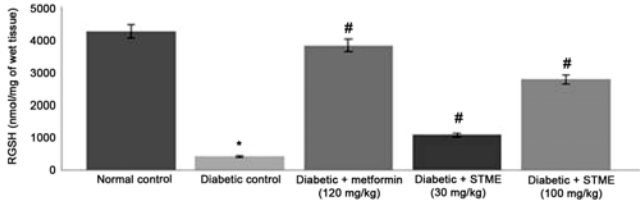

Fig. 12 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on reduced glutathione in blood serum of alloxan-induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to the normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

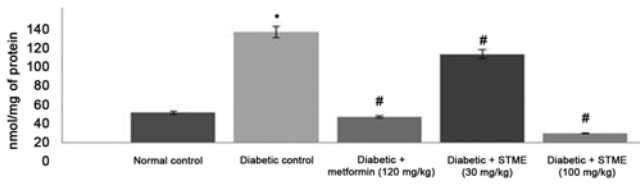

Fig. 13 — Effect of STME (30 and 100 mg/kg) and metformin (120 mg/kg) on thiobarbituric acid levels in the pancreatic homogenate of alloxan-induced diabetic neuropathy in diabetic rats.
N= 6, all data were subjected to ANOVA followed by Dunnett's test, the observations are mean±SEM. *P <0.05 as compared to the normal control group and # P <0.05 as compared to diabetic control group; STME- *Solanum torvum* methanolic extract.

treated with metformin and STME (30 and 100 mg/ kg). The sciatic nerve of normal control rat's showing normal sciatic nerve fibres and normal outer membrane of blood vessel (Plate 1). The diabetic control group showed oedema round the epineurium and infiltration of neutrophils around the blood vessels and showed swelling of nerve fibres (Plate 2).

Diabetic rats treated with metformin (120 mg/kg) showed swelling of nerve fibres and demylination of nerve fibres. (Plate 3). Macrophages and monocytes were observed around the Schwann cells of diabetic rats treated with STME (30 mg/kg), (Plate 4) while diabetic rats treated with STME (100 mg/ kg) showed mild oedema around the epineurium, few infiltrating neutrophils around the blood vessels and only minor swelling of nerve fibres (Plate 5).

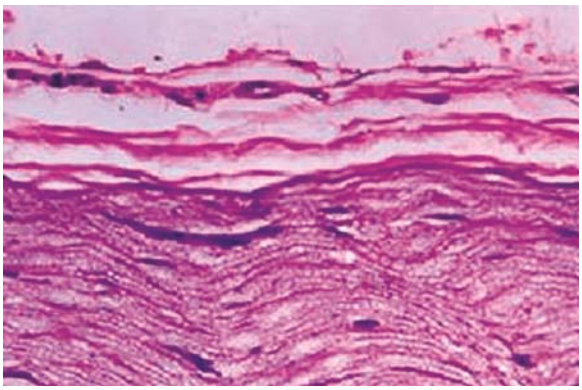

Plate 1 — Section of H&E stained sciatic nerve of normal control rat's showing normal sciatic nerve fibres and normal outer membrane of the blood vessel (100X).

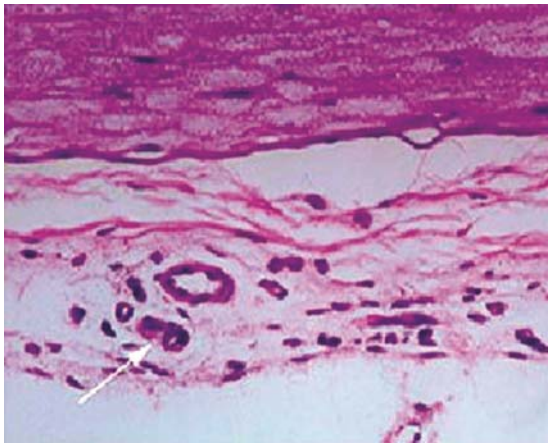

Plate 2 — Section of H&E stained sciatic nerve of diabetic control rats showed oedema around the epineurium and infiltration of neutrophils around the blood vessel and swelling of nerve fibers (100X).

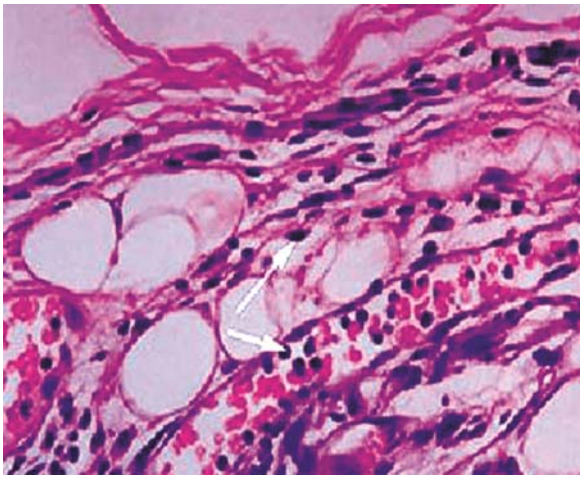

Plate 3 — Section of H&E stained sciatic nerve of diabetic rats treated with metformin (120 mg/kg) showed swelling of nerve fibres and demylination of nerve fibres (100X).

Discussion

The present study shows the effect of *S. torvum* on diabetic neuropathy in alloxan-induced diabetic rats as assessed by its morphological, behavioural, biochemical and histopathological parameters.

Reactive oxygen species (ROS) is a product of biochemical and physiological processes. Environmental stress increases the levels of free radical drastically, thereby disturbing the equilibrium between free radical production and the antioxidant levels resulting in oxidative stress because of excess ROS, antioxidant depletion, or both. With cellular production of ROS, damage to cellular macromolecules such as lipid, protein and DNA mutation in damaged target cells and tissue occurs resulting in cell death. This damage is associated with an increase in the risk of diseases such as diabetes and complications associated with diabetes, such as neuropathies, retinopathies, nephropathy etc[1]. A strong relationship exists between glycaemia and diabetic microvascular complications in both type I and type II diabetes. Generation of superoxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy. Early in the course of diabetes intracellular hyperglycemia causes the abnormality in blood flow with increased vascular permeability. Quantitative and qualitative abnormalities of extra vascular matrix contribute to an irreversible increase in vascular permeability. With time microvascular cell loss occurs resulting in programmed cell death. Hyperglycemia may also affect the production of factors for endothelial and neuronal cell death.

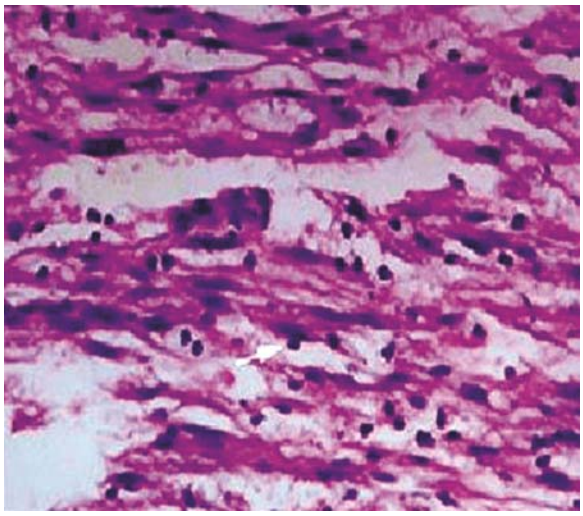

Plate 4 — Section of H&E stained sciatic nerve of diabetic rats treated with STME (30 mg/kg) showed accumulation of macrophages and monocytes around the schwann cells (100X).

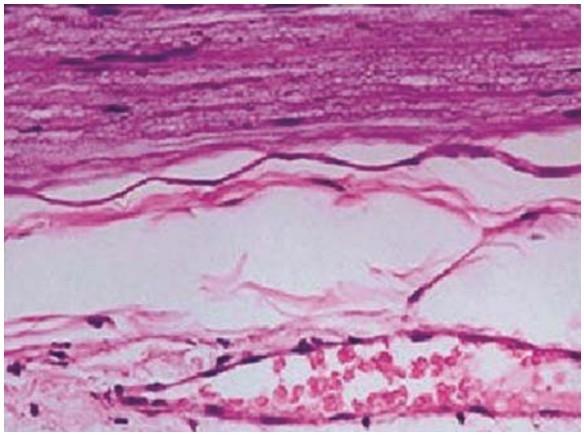

Plate 5 — Section of H&E stained sciatic nerve of diabetic rats treated with STME (100 mg/kg) showed mild oedema around the epineurium and few infiltrating neutrophils around blood vessels and minor swelling of nerve fibres (100X).

Together these changes lead to oedema, ischemia, and hypoxia-induced neovascularization in the retina; proteinuria, mesangial matrix expansion, glomerulosclerosis in the kidney and multifocal axial degradation in peripheral nerves. Generation of super peroxide due to oxidative stress in diabetes may be responsible for vascular and neuronal complications of painful neuropathy. DN is a very common complication of diabetes. Peripheral nerve injury is associated with neuropathic pain and is characterized by sensory abnormalities such as unpleasant abnormal sensation, increased response to painful stimuli and pain in response to the stimulus that does not normally provoke pain and with motor incoordination. Alloxon and it's reduction products dialuric acid, establish a redox cycle with the formation of super peroxide radical. These radicals undergo dismutation to hydrogen peroxide. The action of reactive oxygen species with a simultaneous massive increase in the cystolic calcium concentration causes rapid beta cell destruction[29], hence, alloxan was selected in the present study.

The present study indicated that methanolic extract of *S. torvum* seeds exhibited good antidiabetic and antioxidant activity when administered at the dose 100 mg/kg orally. The high dose of STME (100 mg/kg), showed a similar effect as that of metformin (120 mg/kg, oral hypoglycemic agent). The development of diabetes was observed after 72 hours of induction of diabetes by Alloxan monohydrate (150 mg/kg). The development of neuropathy was observed after 2nd week of induction of diabetes. *In vitro* assay of the present study indicates that percentage uptake of glucose by yeast cells was increased with increasing dose of STME at various concentration of glucose (5, 10, and 25 mM). The STME at a higher concentration that is above 40 µL/mL exhibited glucose uptake activity comparable with standard metronidazole solution. The morphological study such as body weight and water intake was assessed once in a week. Diabetic rats showed significant decrease in body weight as compared to normal control rats. Diabetic rats treated with metformin (120 mg/kg) and STME (30 and 100 mg/kg) showed significant improvement in body weight as compared to diabetic control rats in the 4th and 5th week of treatment schedule. Water intake was decreased in diabetic control rats as compared with normal control, metformin and STME treated diabetic rats.

The behavioural parameters such as hyperalgesia, cold allodynia and motor coordination were assessed by using tail immersion test in hot and cold water, hot plate method, acetone drop test and rota rod test respectively. In behavioural tests, diabetic rats showed a significant reduction in tail and paw withdrawal latencies in tail immersion tests for hot and cold water and hot plate method respectively. A significant decrease in reaction time for acetone drop method and a significant decrease in fall off time for rota rod test was observed in diabetic rats as compared to normal control.

In case of tail immersion test in cold and hot water, diabetic rats treated with metformin (120 mg/kg) and diabetic rats treated with STME (100 mg/kg) showed

significant improvement in tail withdrawal latency in 3rd, 4th and 5th week of treatment schedule as compared to diabetic control rats. In case of cold allodynia as assessed by acetone drop test, diabetic rats treated with metformin (120 mg/kg) showed a significant decrease in reaction time at 3rd, 4th, and 5th week of treatment schedule. Diabetic rats treated with STME (100 mg/kg) showed a significant decrease in reaction time at 3rd and 4th week of treatment schedule. As evident from the results of the present study, five-week treatment with STME (100 mg/kg) improved cold allodynia and thermal hyperalgesia in experimental animals.

Motor incoordination was assessed by using rota rod test. Diabetic rats treated with metformin (120 mg/kg) and STME (30 and 100 mg/kg) showed significant improvement in motor coordination as indicated by a decrease in fall off time as compared to diabetic control rats after 2nd week of treatment schedule as compared to the diabetic control rats, thus treatment with STME, prevents motor incoordination in the diabetic rats.

Heat hyperalgesia assessed by hot plate method, diabetic rats treated with metformin (120 mg/kg) and STME (100 mg/kg) showed significant improvement in paw withdrawal latency at 3rd, 4th, and 5th week of treatment schedule as compared to diabetic control rats. After treatment, blood glucose levels significantly decreased in diabetic rats treated with metformin (120 mg/kg) and diabetic rats treated with STME (100 mg/kg) as compared to diabetic control rats.

There was a significant decrease in relative organ weight of pancreas of animals of diabetic control rats as compared with normal control rats, and a significant increase in relative organ weight of pancreas in diabetic rats treated with standard metformin (120 mg/kg) and in diabetic rats treated with STME (30 and 100 mg/kg) as compared to diabetic control rats. The purpose of relative organ weight analysis is to detect any direct treatment effect on the organ weights over and above any indirect effects caused by the effects of the treatment on body weight.

Thiobarbituric acid reactive substances are formed as a byproduct of lipid peroxidation (degradation products of fats) which can be detected by TBARS assay using Thiobarbituric acid as a reagent. Since reactive oxygen species have short half-lives they are difficult to measure directly, so that it can be measured by several products of damage produced by oxidative stress, such as TBARS. Assay measures malonaldehyde present in the sample, as well as malonaldehyde generated from lipid hydro peroxidase by the hydrolytic conditions of the reaction[5,30].

Glutathione (GSH) is highly abundant in all cell compartments and is the major soluble antioxidant. Reduced GSH/Oxidized GSH ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways. It detoxifies hydrogen peroxide and lipid peroxides via the action of GSH-Peroxidase[30].

RGSH is a primary antioxidant in the cell. Significant decrease in the amount of RGSH was observed in the diabetic control group compared with a normal control group, while in diabetic rat treated with metformin and STME (30 and 100 mg/kg) showed a significant increase in RGSH levels as compared with the diabetic control group. In case of TBARS, there was a significant increase in TBARS levels in the diabetic control rats as compared to normal rats, while diabetic rats treated with STME (30 and 100 mg/kg) showed a significant decrease in TBARS levels as compared with diabetic control rats, indicating the antioxidant activity of STME.

We observed that the sciatic nerve of diabetic rats produced severe pathological changes as compared with groups treated with metformin and STME (30 and 100 mg/ kg). The diabetic control group showed oedema round the epineurium and infiltration of neutrophils around the blood vessels and showed swelling of the nerve. Diabetic rats treated with metformin (120 mg/kg) showed swelling of nerve fibres and demylination of nerve fibres, while diabetic rats treated with STME (100 mg/ kg) showed mild oedema around the epineurium with few infiltrating neutrophils around the blood vessels and only minor swelling of nerve fibres, thus STME treated animals showed sciatic nerve stability than diabetic control animals.

The present study has shown the effect of *S. torvum* on alloxan-induced diabetic neuropathy in diabetic rats by assessing its morphological, behaviour, antioxidant and histopathological studies. Treatment with *S. torvum* (100 mg/kg) in diabetic rats showed significant improvement in tail withdrawal latency in case of a hot plate, tail immersion in hot and cold water, and increase in reaction time for acetone drop test and improvement in motor coordination assessed by rota rod test. Significant increase in relative organ weight was observed in STME (100 mg/kg) treated group. Increase in RGSH and decrease in TBARS levels were observed in STME (100 mg/kg) treated groups. STME (100 mg/kg) has also reversed histopathological changes as seen in diabetic control animals.

Conclusion

Thus it can be concluded that the methanolic extract of *S. torvum* (100 mg/kg) has a neuroprotective, antioxidant, and antidiabetic effect against alloxan-induced diabetic rats.

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