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# OLEANANE-TYPE TRITERPENOID SAPONIN OF *MOMORDICA CYMBALARIA* EXHIBITS NEUROPROTECTIVE ACTIVITY IN DIABETIC PERIPHERAL NEUROPATHY BY AFFECTING THE POLYOL PATHWAY

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**Keywords:**

Diabetic peripheral neuropathy (DPN), *Momordica cymbalaria*, Nerve conduction velocity, aldose reductase, sorbitol dehydrogenase, sorbitol.

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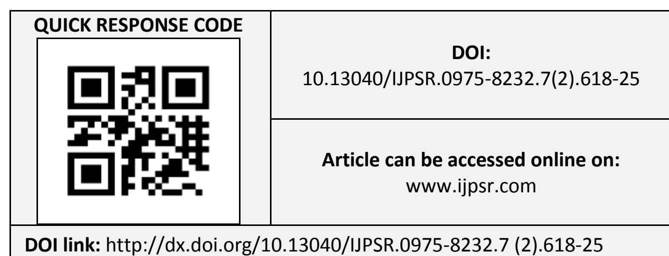
**ABSTRACT:** *Momordica cymbalaria* Fenzl (Cucurbitaceae) fruit powder and extracts are reported to have Type 1 antidiabetic activity in experimental diabetic models. The objective of this study was to investigate the neuroprotective action of an oleanane-type triterpenoid saponin isolated from the plant in diabetic peripheral neuropathy (DPN) by *in vivo* and *in vitro* methods. Male Wistar rats were rendered diabetic by administration of streptozotocin intraperitoneally. The rats were maintained for six weeks to induce neuropathy. Treatment groups received the saponin and epalrestat, an aldose reductase inhibitor. At the end of treatment, the animals were evaluated for various parameters of neuropathy like muscular grip strength and pain sensation tests using hot plate and tail-flick methods, and nerve conduction velocity (NCV) measurement. *In vitro* models of DPN were created by initiating sciatic nerve cultures of rats in 12-well plates. The nerve cultures were then rendered diabetic by exposing them to high glucose. After incubation of 7 days, the tissues were assayed for aldose reductase (AR) and sorbitol dehydrogenase (SDH) activities. Intraneuronal accumulation of sorbitol was also measured using HPLC method. The saponin exhibited significant reduction in the AR and SDH activities, reduction in the former being more prominent. Sorbitol accumulation in the nerves was also found to be less as compared to glucose control. Improvement of muscular grip strength, reaction time to pain sensation and nerve conduction velocity was demonstrated by the treated group. The results suggest that the saponin possesses significant neuroprotective activity and may prove to be good therapeutic agent for managing and treating diabetic neuropathy.

**INTRODUCTION:** Diabetic peripheral neuropathy is a neurological disorder that is associated with diabetes mellitus. This condition is thought to arise from injury of the microvasculature that innervates the nerves (vasa nervorum) in addition to conditions of the macrovessels.



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Diabetic neuropathy influences all peripheral nerves including sensory and motor neurons and the autonomic nervous system. As all the organs and systems are innervated, it can affect all.

The polyol pathway of glucose metabolism plays a crucial role in the development of neuropathy. It becomes active when intracellular glucose levels are elevated. [1] It is a two-step metabolic pathway in which glucose is reduced to sorbitol by the enzyme aldose reductase, which is then converted to fructose by sorbitol dehydrogenase. The first enzyme, aldose reductase (AR), requires NADPH as co-factor, and the second enzyme sorbitol

dehydrogenase (SDH) needs $NAD^+$. During hyperglycemia, sorbitol accumulates in

AR-containing tissues, presumably because oxidation by SDH is rate limiting and because polyols do not readily diffuse across cell membranes it was thought that accumulation of sorbitol produces osmotic that leads to diabetic lesions. [2, 3] In animal models, treatment with AR inhibitors (ARI) was shown to be effective in preventing the development of various diabetic complications, including cataract, neuropathy, and nephropathy. [4] Alternately, it has been suggested that exaggerated SDH activity may produce a state of "pseudohypoxia" by altering the NADH-to-NAD+ ratio.[5] Although SDH inhibitors (SDIs) have been used to address the latter possibility conflicting results have been obtained. [6, 7] The polyol pathway has also been known to induce oxidative stress in tissues through three potential mechanisms, (i) AR activity depletes its co-factor NADPH, which is also required for glutathione reductase to regenerate GSH. Under hyperglycemic condition, as much as 30% of the glucose is channeled into the polyol pathway, [8] causing a substantial depletion of NADPH and consequently a significant decrease in the GSH level.

Thus, during hyperglycemia, AR activity diminishes the cellular antioxidant capacity, (ii) oxidation of sorbitol to fructose by SDH causes oxidative stress because its co-factor $NAD^+$ is converted to NADH in the process, and NADH is the substrate for NADH oxidase to generate ROS,[9] and (iii) the polyol pathway converts glucose to fructose. Because fructose and its metabolites fructose-3-phosphate and 3-deoxyglucosone are more potent non-enzymatic glycation agents than glucose, the flux of glucose through the polyol pathway would increase advance glycation end products (AGE) formation.

Plants constitute a rich source of bioactive chemicals. Since many plants are largely free from adverse effects and have excellent pharmacological actions, they could possibly lead to the development of new classes of safer antidiabetic agents or diabetic complication resolving agents. *Momordica cymbalaria* (Cucurbitaceae) has been found to possess Type I [10] and Type II [11] anti-diabetic activities. A triterpenoid saponin of oleanane-type has been isolated from the roots of *Momordica cymbalaria* (SMC) which has also been reported to possess neuroprotective activity in streptozotocin-induced diabetic mice. [12] The current study was conducted to investigate whether SMC interacts with the polyol pathway in hyperglycemic state and thereby exerts its neuroprotective activity.

## MATERIALS AND METHODS:

### Extraction and isolation of saponin:

The oleanane-type triterpenoid saponin was extracted from the roots of *Momordica cymbalaria*.[12]

### Chemicals:

Streptozotocin (Sigma Aldrich), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), glycine, EDTA, Glucose, antibiotics, insulin, D-fructose, Triethanolamine, β-Nicotinamide Adenine Dinucleotide, reduced form (β-NADH), Bovine Serum Albumin (BSA), Sodium phosphate buffer, NADPH, Lithium sulphate, DL-glyceraldehyde, sorbitol and acetonitrile were obtained from Hi-Media Laboratories Ltd., Mumbai.

### Animals:

Male Wistar rats weighing between 160-220 g were maintained in standard laboratory conditions at room temperature (25±2 $^0$C) with 12:12 h L:D. The animals were given pellet chow and water ad libitum except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) at Karnataka College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

### Induction of diabetes:

Diabetes was induced in 16 h fasted animals by intraperitoneal injection of 65 mg/kg body weight of streptozotocin by dissolving in 0.1 M cold sodium citrate buffer (pH 4.5) immediately before use. The animals were allowed free access to 5% w/v glucose solution for the next 24 h to prevent hypoglycemia. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages.

Diabetic animals were validated with the detection of high blood glucose level (>180 mg/dL) 72 hours after the STZ administration. The diabetic animals were continuously fed with a high-fat & high-sugar diet for another six weeks to induce diabetic neuropathy. The DPN model was validated by the lowering of nerve conduction velocity (NCV <40 m/s). The animals were divided into **five** groups each consisting of six rats.

**Group 1:** Administered vehicle serves as Normal control.

**Group 2:** Administered Streptozotocin (65 mg/kg i.p.) - Serves as diabetic peripheral neuropathy (DPN) control.

**Group 3:** Administered reference standard Insulin (4U/kg i.p.).

**Group 4:** DPN rats treated with SMC (100 mg/kg, p.o) [13]

**Group 5:** DPN rats treated with epalrestat (100 mg/kg, p.o.) [14]

**Muscular grip strength test using Rota rod method:**
The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using inclined plane or rotating rods. The difference in the fall off time from the rotating rod between the control and treated animal is taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod is adjusted such that a normal animal can stay on the plane or on the rod for an appreciable period (3-5min) of time. The animals underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test.[15]

**Pain sensation test using Tail flick method:**
Before initiating the test, the tail of each animal was dipped in water at 29 $^0$C for 30 mins. Then the whole tail was submerged water at 49 $^0$C. The time taken for the animal to show a characteristic tail flick response was recorded. The test was repeated three times for each animal and the average was considered as the withdrawal latency for each animal.[16]

**Pain sensation test using Hot plate method:**
In this method heat is used as a source of pain. Animals are individually placed on a hot plate maintained at constant temperature (55 $^0$C) and the reaction of animals such as paw licking or jump response is taken as the end point. Normally animals show response in 6-8sec. A cut off period of 15 sec in observed to avoid damage to the paws. Prior to any treatment, the animals were allowed to familiarize with the test procedure and apparatus, and baseline values were obtained.[17]

**Measurement of Nerve Conduction Velocity (NCV):**
After anesthesia, rat backs were shaved and motor NCV was recorded in a temperature-controlled environment from the left sciatic tibial nerve by a modified non-invasive method. [18] Briefly, the rectal temperature was maintained at 37 °C, and the left sciatic nerve was stimulated proximally at the sciatic notch and distally at the knee via bipolar electrodes by AD Instruments (Powerlab data acquisition system, New Zealand). The compound muscle action potential (CMAP) was recorded from the ankle by unipolar pin electrodes. NCV was calculated as the ratio of the distance in millimeters between both sites of stimulation divided by the difference between proximal and distal latencies measured in milliseconds, giving a value for NCV in meters per second (m/s).

**Sciatic nerve culture:**
Adult male Wistar rat (10 weeks old) was deeply anesthetized with a combination of ketamine (50 mg/kg) and xylazine (2.6 mg/kg). The animal's left sciatic nerve was exposed through a dorsal incision of the thigh and the nerve of full length was removed and transferred to a petri dish containing DMEM. The nerves were de-myelinated by stripping off the epineurium with a pair of sterile forceps and cut into two equal halves. Each piece was kept in each well of a 12-well plate coated with 1% collagen peptide hydrolysate of Type I collagen (Himedia, Mumbai). The pieces were covered with a drop of fetal bovine serum (FBS) and incubated over night at 37 $^0$C in 5% $CO_2$ to facilitate adherence on to the surface. After 24 h 1ml of fresh

DMEM supplemented with 10% FBS, Penicillin G (100 IU/ml), Streptomycin sulfate (100μg/ml) and Amphotericin B (2.5 μg/ml) was added to all the wells and incubated. The nerve cultures were grouped as follows:

**Group 1:** Normal control – nerve in glucose-free medium

**Group 2:** High glucose control – nerve in high glucose (56 mM) medium

**Group 3:** Treated group – high glucose control + 500 μg/ml SMC

**Group 4:** Standard group – high glucose control + 1μM epalrestat [19]

The cultures were incubated for six days at 37 $^0$C in 5% $CO_2$ atmosphere. After incubation the nerves were homogenized with a Polytron homogenizer using a lysis buffer at 0-4 $^0$C containing 10 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton-X-100, 1% NP40 and a protease inhibitor cocktail of aprotinin, benzamidine, leupeptin, pepstatin A, and PMSF. The lysate was centrifuged and the supernatant was collected and stored at -20 $^0$C until used. The supernatant was estimated for aldose reductase (AR) and sorbitol dehydrogenase (SDH) activities and, sorbitol content by HPLC.

**Aldose reductase (AR) activity:**
For the determination of the sciatic nerve AR activity, 0.7 mL of phosphate buffer (0.067 M), 0.1 mL of NADPH ($25\times10^{-5}$ M), 0.1 mL of homogenate supernatant, 0.1 mL of DL-glyceraldehyde (substrate) ($5\times10^{-4}$ M) were taken in a cuvette. Absorbance of the final solution was taken against a reference cuvette containing all components except the substrate, DL-glyceraldehyde. The enzymatic reaction was started by the addition of the substrate and the absorbance (OD) was recorded at 340 nm for 3 min at 30 s interval. AR activity was expressed as μmoles/min/ml. [20]

**Sorbitol dehydrogenase (SDH) activity:**
For the determination of the sciatic nerve SDH activity, 2.35 ml of 100 mM Triethanolamine buffer pH 7.6, 0.5 ml of 1.1 M D-fructose solution and 0.05 ml of 12.8 mM β-NADH solution were added to a cuvette and mixed by inversion to equilibrate to 25 $^0$C. Using a thermostatted spectrophotometer, the $A_{340nm}$ is monitored until constant and, then 0.1 ml of the supernatant was added to start the reaction. A reference cuvette was maintained containing all components except 1% BSA in place of the homogenate supernatant. The decrease in $A_{340nm}$ was recorded for 5 mins at 30 s interval. SDH activity was expressed as μmoles/min/ml. [21]

**Chromatography of sorbitol:**
The separation and quantification of samples and standards were performed using a Agilant 1120 binary HPLC pump equipped with a dual wavelength absorbance detector, and a EZChrome software. Separations were performed on a Waters Sunfire® C18 reversed-phase column (250mm×4.6mm, 5μm, Milford, MA) and peak detection was performed at 231 nm. A gradient elution using $H_2O$ and acetonitrile and, a flow rate of 1.0 ml/min was maintained throughout the separation. Injection volume used was 25 μL. Sorbitol reference standard (Sigma-Aldrich, India) was used of concentration 100 μg/ml. An initial solvent composition of 30% $H_2O$ and 70% acetonitrile was maintained at injection and held for 2 min. At 2 min the proportion of acetonitrile was increased linearly to 87.5% over a 2 min period then held for 2 min. At 4 min the proportion of acetonitrile was increased linearly to 95% over 1 min and held for 1 min. At 5 min, the elution solvent was returned to 87.5% over a 1 min period followed by to 80% for the next 1 min and then to 70% in the next one min. The system is allowed to equilibrate for 5min prior to starting the next injection.[22]

## RESULTS:

**Muscular grip strength test:**
The measure of muscular grip strength is expressed in terms of the time of fall or time of residence of the animal from the rod (**Table 1**). In diabetic control group, muscular grip strength was significantly ($p < 0.001$) decreased as compared to normal control rats. The treatment of diabetic rats showed a significant ($p < 0.001$; $p < 0.05$) improvement in the muscular grip strength as compared to diabetic control rats.

**TABLE 1: EFFECT OF SAPONIN OF *MOMORDICA CYMBALARIA* (SMC) ON TIME OF RESIDENCE/FALL**

| Sl. No. | Group | Time of residence/fall (sec) |
|---|---|---|
| 1 | Normal control | 30.4 ± 0.545 |
| 2 | DPN control | 10.6 ± 0.257*** |
| 3 | Insulin-treated | 28.38 ± 0.221### |
| 4 | SMC-treated | 23.07 ± 0.168### |
| 5 | Epalrestat-treated | 20.30 ± 0.230### |

Values are expressed as Mean ± S.E.M (n=6)
***P<0.001 when compared with normal control
###P<0.001 when compared with DPN control

### Pain sensation test by Tail flick and Hot plate methods:

Treated diabetic control rats showed significant (P < 0.001) increase in the tail-flick latency time and a significant (P < 0.001) decrease in the response time with hot-plate method when compared with normal control (group 1). The groups receiving insulin, SMC and epalrestat (groups 3, 4 and 5) showed significant (P < 0.001) increase in the tail-flick latency time and a statistically significant (P < 0.001) decrease in the response time with hot-plate method when compared with the diabetic control group (**Table 2**).

**TABLE 2: EFFECT OF SAPONIN OF *MOMORDICA CYMBALARIA* (SMC) ON TAIL-FLICK LATENCY AND HOT PLATE RESPONSE TIME**

| Sl. No. | Group | Tail-flick latency (sec) | Hot plate response time (sec) |
|---|---|---|---|
| 1 | Normal control | 3.192 ± 0.0460 | 0.612 ± 0.0087 |
| 2 | DPN control | 10.28 ±0.0688*** | 3.23 ± 0.0166*** |
| 3 | Insulin-treated | 4.20± 0.0070### | 1.13 ± 0.0602### |
| 4 | SMC-treated | 3.29± 0.0114### | 0.715 ± 0.0099### |
| 5 | Epalrestat-treated | 5.14± 0.0154### | 1.482 ± 0.0087### |

Values are expressed as Mean ± S.E.M (n=6).
***p<0.001 when compared with normal control.
###p< 0.001 when compared with DPN control.

### Nerve Conduction Velocity (NCV):

Diabetic peripheral neuropathy is characterized by lowering of NCV. The NCV in different groups were measured after the treatment with SMC, insulin, and epalrestat. It was found that animals with DPN had significantly lower NCV compared with control animals (P <0.001). DPN control group showed significantly lower NCV compared to the normal control group. Insulin, SMC and epalrestat treated group showed significant recovery in NCV compared with the DPN group (p <0.0001). Similar results were obtained when the CMAP was measured (**Table 3**).

**TABLE 3: EFFECT OF SAPONIN OF *MOMORDICA CYMBALARIA* (SMC) ON NERVE CONDUCTION VELOCITY (NCV) AND COMPOUND MUSCLE ACTION POTENTIAL (CMAP)**

| Sl. No. | Group | NCV (m/s) | CMAP (mV) |
|---|---|---|---|
| 1 | Normal control | 43.87 ± 0.352 | 15.13 ± 0.131 |
| 2 | DPN control | 23.73 ± 0.243*** | 9.042 ± 0.142*** |
| 3 | Insulin-treated | 28.28 ± 0.323### | 10.37 ± 0.210### |
| 4 | SMC-treated | 42.39 ± 0.251### | 12.94 ± 0.221### |
| 5 | Epalrestat-treated | 43.33 ± 0.220### | 14.18 ± 0.232### |

Values are expressed as Mean ± S.E.M (n=6)
***P<0.001 when compared with normal control
###P<0.001 when compared with DPN control

### Aldose reductase (AR) and Sorbitol dehydrogenase (SDH) activities in sciatic nerve:

Saponin of *Momordica cymbalaria* has shown significant AR inhibition activity (p<0.001) when compared to high glucose control (group 2). The AR inhibition activity of SMC is, in fact, comparable to the activity of epalrestat, the standard AR inhibitor. SMC also inhibited SDH activity as compared to the controls. However, this inhibitory activity was not found to be significant. Epalrestat too did not show any significant SDH inhibition activity (**Table 4**). Aldose reductase and SDH activities for the normal control without glucose were not detected.

**TABLE 4: EFFECT OF SAPONIN OF *MOMORDICA CYMBALARIA* (SMC) ON THE ACTIVITIES OF ALDOSE REDUCTASE (AR) AND SORBITOL DEHYDROGENASE (SDH) IN SCIATIC NERVE**

| Sl. No. | Group | AR activity (µmoles/min/ml) | SDH activity (µmoles/min/ml) |
|---|---|---|---|
| 1 | Normal control (without glucose) | 0.000 | 0.000 |
| 2 | High glucose control (56 mM) | 0.0257 ± 0.00000882*** | 0.0096 ± 0.0000281*** |
| 3 | Treated group (high glucose control + 500 µg/ml SMC) | 0.0108 ± 0.0000107### | 0.0088 ± 0.0000298 |
| 4 | Standard group (high glucose control + 1µM epalrestat) | 0.0102 ± 0.000008819### | 0.0094 ± 0.0000313 |

Values are expressed as Mean ± S.E.M (n=6)
***P<0.001 when compared with normal control
###P<0.001 when compared with high glucose control

**Measurement of intraneural sorbitol:**

Saponin of *Momordica cymbalaria* has significantly reduced the formation of sorbitol (p<0.001) in the sciatic nerve under hyperglycemic state when compared to high glucose control group. The activity has been found to comparable to that of the standard drug epalrestat. However, sorbitol remained undetected in the normal control group without glucose (**Table 5**).

**TABLE 5: EFFECT OF SAPONIN OF MOMORDICA CYMBALARIA (SMC) ON INTRANEURAL ACCUMULATION OF SORBITOL**

| Sl. No. | Group | Log AUC | Concentration of sorbitol (µg/ml) |
|---|---|---|---|
| 1 | Normal control (without glucose) | --- | --- |
| 2 | High glucose control (56 mM) | 7.907 ± 0.0125 | 93.45 ± 0.1482*** |
| 3 | Treated group (high glucose control + 500 µg/ml SMC) | 4.891 ± 0.0015 | 57.8 ± 0.0181### |
| 4 | Standard group (high glucose control + 1µM epalrestat) | 3.472 ± 0.0033 | 41.04 ± 0.0394### |

Values are expressed as Mean ± S.E.M (n=6)
***P<0.001 when compared with normal control
###P<0.001 when compared with high glucose control

**DISCUSSION:** The results of this study demonstrate an ameliorating effect of the saponin of *Momordica cymbalaria* in diabetic peripheral neuropathy. In the muscular grip strength test the animals exhibited increased residence time on the Rotarod in contrast to the diabetic animals. Increase in glycosylation of proteins including hemoglobin is seen with uncontrolled or poorly controlled diabetes. The glycation of myelin protein may contribute to the impairment of nerve conduction. These advanced glycation end products are also present in peripheral nerves which could interfere with axonal transports. [23]

There is loss of pain perception in diabetes probably due to nerve damage and induction of peripheral neuropathy. [24, 25] Thermal hypoalgesia has been reported in diabetic rats using the tail-flick test or the hotplate test. [26, 27] In the pain sensation tests using tail-flick and hot-plate methods the animals showed improvement in response towards the pain stimuli as compared to the diabetic animals, the responses being comparable to the insulin and standard treated groups, suggesting protective effect against neuropathy.

Diabetic peripheral neuropathy is marked by impaired nerve conduction velocity (NCV) and loss in unmyelinated and myelinated peripheral nerve fibers due to hyperglycemia, abnormal fatty acid metabolism, ischemic hypoxia, and/or oxidative stress. [28] Also low peripheral nerve conduction velocities and amplitudes are strongly related to diabetic microvascular complications in type 1 diabetes. [29]

In this study, treatment with SMC has significantly improved the NCV and CMAP in the sciatic nerves of animals. This response is comparable to that of the response provided by epalrestat, an aldose reductase inhibitor, which in turn is almost equal to that of the normal control. The reversal of NCVs to normal upon treatment may be implicated to improvement of endoneurial blood flow thereby reversing endoneurial ischemia and ROS-mediated damage in the peripheral nerve tissues.[28]

The polyol pathway is a crucial metabolic pathway in the metabolism of glucose in a hyperglycemic state. When glucose is in excess it is shunted into the pathway where it is converted by its first rate-limiting enzyme aldose reductase to which is then converted to fructose by sorbitol dehydrogenase. Sorbitol is an alcohol, polyhydroxylated, and strongly hydrophilic, and therefore does not diffuse readily through cell membranes and accumulates intracellularly with possible osmotic consequences. [1] The fructose produced by the polyol pathway can become phosphorylated to fructose-3-phosphate [30, 31], which is broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that enter in the formation of advanced glycation end products (AGEs). [30] The usage of NADPH by AR may result in less cofactor available for glutathione reductase, which is critical for the maintenance of the intracellular pool of reduced glutathione (GSH).

This would lessen the capability of cells to respond to oxidative stress. [32] The saponin of *Momordica cymbalaria* has been found to reduce the increased activity of aldose reductase in treated nerves when compared to the high glucose control. The AR

inhibition activity of SMC was found to be nearer to that of the marketed AR inhibitor, epalrestat, proving its AR inhibiting potential. Aldose reductase over-expression is implicated in the development of diabetic neuropathy and it is ameliorated by the use of AR inhibitors. [33] This over-expression of AR has been found to be involved in the nuclear translocation of inflammatory nuclear factor-κB (NF-κB) and activation of protein kinase C (PKC) [34] which leads to tissue inflammation. Treatment of vascular smooth muscle cells with the aldose reductase inhibitors tolrestat and sorbinil prevented high glucose–induced protein kinase C (PKC) activation and nuclear translocation of NF-κB.[35] Under euglycemic conditions, the polyol pathway represents a minor source of glucose utilization, accounting for <3% of glucose consumption. [36]

This may be the underlying cause as to why the sorbitol dehydrogenase activity was not found to be significant in the high glucose control group. The SDH inhibition activity of SMC was not found to be significant as that of its AR inhibition activity. The reason may be due to its non-specificity towards sorbitol dehydrogenase. In fact inhibition of sorbitol dehydrogenase does not offer an effective approach for prevention of oxidation and metabolic imbalances in the peripheral nerve that is induced by diabetes and is adverse rather than beneficial. [37]

Glucose flux into the polyol pathway due to hyperglycemia leads to formation of sorbitol. This accumulates in the tissue as it is impermeable and produces hyperosmotic stress that causes tissue injury. Aldose reductase inhibitors have shown to reduce the levels of sorbitol in AR-dependent tissues like lens epithelial cells, nerves, vascular smooth muscle cells, endothelial cells, retinal pericytes, seminal vesicles and proximal tubular epithelial cells of the kidneys. [35] Our study shows that SMC has markedly prevented the accumulation of sorbitol in the high glucose-induced diabetic sciatic nerves. This is in absolute agreement with the findings of AR inhibition experiment. The exact mechanism of neuroprotection by SMC still needs to be investigated. Its role in the formation of advanced glycated end products (AGEs), activation of NF-κB, over-expression of PKC and inflammatory cytokines, and oxidative stress has to be established, which opens a scope for further extensive research.

**CONCLUSION:** In conclusion the study reveals the neuroprotective activity of the saponin of *Momordica cymbalaria* in terms of improvement in muscular grip strength, reaction to heat and pain, and reversal of nerve conduction towards normal. The saponin has also shown to inhibit aldose reductase efficiently which is comparable to the action shown by the standard drug epalrestat, thereby reducing the accumulation of sorbitol in the nerves.

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