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Original Article

Effect of Topiramate on Mitochondrial Biogenesis and Neuroinflammation in Chronic Constriction Injury and Streptozotocin-induced Peripheral Neuropathy

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ABSTRACT

Background: Because neuropathic pain is currently a poorly understood medical condition, there is a need to identify a more effective therapeutic drug with a low toxicity profile. **Purpose:** To address the behavioural, oxidative stress, and cytokine-mediated inflammatory pathways involved in sciatic nerve, the current study protocol used Chronic Constriction Injury (CCI) and Streptozotocin (STZ) induced neuropathic pain methods. **Materials and Methods:** Following CCI to the sciatic nerve and administration of STZ 60mg/kg, rats develop painful neuropathy characterized by hyperalgesia, allodynia, oxidative stress, neuroinflammation, and increased total calcium levels. **Results:** Topiramate (20, 40, and 80 mg/kg) treatment for two weeks beginning on the 15th day of CCI surgery and ending on the 29th day of diabetes confirmation significantly and dose dependently reduced the development of allodynia and hyperalgesia. Furthermore, topiramate treatment produced significant dose-dependent antioxidant and anti-inflammatory effects by restoring the balance of oxidative stress and decreasing the levels of inflammatory markers such as TNF-α, IL-1, and IL-6, as well as total calcium in sciatic nerve homogenate. **Conclusion:** To summarize, the treatment with different doses of topiramate produces analgesic, antioxidant, and anti-inflammatory effects in a dose dependent manner, owing to the increased curiosity to understand the underlying pain producing mechanism and its translation into signs and symptoms of CCI and STZ induced neuropathy.

Keywords: Topiramate, CCI, STZ, Oxidative Stress, Cytokines.

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INTRODUCTION

International Association of pain put forward the new concept of neuropathic pain as a chronic medical condition with an impaired function of somatosensory nerves.[1] Initial findings of clinical-based physical observation reveal the estimated 1% to 3% populations were affected and about 6.5% to 17.9% of general people are suffering from neuropathic pain.[2] Currently, the epidemiological data showed that almost 6.9% to 10% of individuals are suffered from neuropathic pain.[3] Neuropathic pain is a hallmark of different behavioral patterns like hyperalgesia and allodynia, and diseases like cancer, diabetes, HIV infections, and cervical radiculopathy, play a vital role in the genesis of neuropathic pain.[4] Due to difficulty in doing a clinical screening of a huge number of populations, and the undeveloped gold standard for screening methods, neuropathic pain is considered a heterogenic medical condition.[5] The pain that arises from neuropathy and the nociceptive pathway is different, therefore it requires different treatment guidelines creating difficulty for the researchers in its management.[6] Numerous laboratory reports are available for the pharmacology-based treatment approach, but currently, agents such as anti-depressants like tricyclic and serotonin-norepinephrine reuptake inhibitors and antiepileptics like gabapentin and pregabalin are used as a first-line treatment option.[7]

The mutual relationship between oxidative stress and nerve inflammation is responsible for the development of neuropathic pain.[8] Initially, under the stress of nerve injury, the inflammatory pathways are activated, and as a consequence of this, the potential pro-inflammatory cytokines like tumor necrosis factor – α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are released.[9] Moreover, mitochondrial production of Reactive Oxygen Species (ROS) induces inflammation, nerve stimulation, and proteins degradation, to trigger apoptotic pathways-induced neuronal death.[10] Henceforth, developing the mitochondrial protective approach against ROS is necessary to manage the pathogenesis of neuropathic pain. Extensive studies revealed

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that TNF-α decreases the threshold potential for stimulation of nociceptors and increases the pain sensitivity of sensory neurons that leads to the development of hyperalgesia and allodynia. Henceforth, it is the need of the hour to search for better therapeutic strategies which not only resolve the influence of inflammation but also control the level of ROS. Also, there is a great need to find an agent with modified efficiency and a greater safety profile for the treatment of neuropathic pain.

Fortunately, the underlying mechanism involved in the pathogenesis of epilepsy and neuropathic pain follows a similar pattern of events characterized by exaggerated stimulation of neurons.[11] Currently, newer antiepileptic agents like gabapentin and topiramate have been extensively tested in different neurodegenerative disorders as they possess unique Na^+ and Ca^{2+} channel blocking properties, and are approved agents for the monotherapy of peripheral neuropathy.[12] Topiramate is weak carbonic anhydrase inhibitor, and its neuroprotective potential was proven in simple or complex partial seizures, primary generalized seizures, and Lennox-Gastaut syndrome in children.[13] Fundamentally, it causes blockage of both voltage-operated Na^+ and Ca^{2+} channels, and AMPA / kainate glutamate receptors and stimulates Gamma-Aminobutyric Acid A ($GABA_A$) mediated Cl^- influx.[14] Moreover, it produces an antioxidant effect by increasing glutathione concentration, reducing lipid peroxidation, and decreasing Nitric Oxide (NO) concentration.[15] The present study takes an account of the beneficial neuroprotective potential of topiramate in animal models of neuropathic pain to explore its dose-dependent analgesic, antioxidant, anti-inflammatory, and neuroprotective effects.

MATERIALS AND METHODS

Drugs and chemicals

Topiramate (Alembic Pharmaceutical Limited, Baroda, India), gabapentin and pregabalin (gift sample, MS University, Baroda, India), streptozotocin (Sigma Aldrich, USA), and sodium pentobarbital (Abbott, India) were obtained as gift samples. All the chemicals and reagents used in the study were purchased from the reputed supplier and of standard grade.

Preparation of drugs solution

The normal saline solution was used for the preparation of gabapentin, and pregabalin[16] and one drop of 10N HCl was added to the normal saline solution of topiramate.[17] The fresh 0.1M citrate buffer with pH 4.5 was used for the preparation of Streptozotocin (STZ).[18] The freshly prepared solution of drugs were used throughout the study protocol.

Experimental animals

The male Wister rats (180-200g) were kept in 12-hr light/dark cycle and maintained at a temperature of 23±2°C, relative humidity of 55% ± 10%. The prior approval for the present study protocol was taken from Institutional Animal Ethics Committee of Shriman Sureshdada Jain College of Pharmacy, Chandwad, Nashik, India (SSDJ/IAEC/2017/01).

Induction of neuropathic pain by Chronic Constriction Injury (CCI)

An injury to the left sciatic nerve was given as per the procedure described by Bennett and Xie (1988).[19] Intraperitoneal injection of sodium pentobarbital (45 mg/kg) was used to anesthetize the rat[20] and the sciatic nerve was loosely tied with four ligatures (4.0 chromic gut, Johnson and Johnson) keeping about 1 mm spacing between each tied knot.[21] After surgery, an incision was then sutured and the individual rat was placed in a separate cage for further study to examine behavioral parameters. After two weeks of surgery i.e. basal, the rats were treated with intraperitoneal topiramate at various doses like 20, 40, and 80 mg/kg body weight/day for the next two weeks. In the present study, the seven groups of rats were made each containing 10 rats (n = 10) as mentioned below.

Group 1: Normal control + treated with normal saline for two weeks.

Group 2: Sham-operated + treated with normal saline for two weeks.

Group 3: CCI + treated with normal saline for two weeks.

Group 4: CCI + gabapentin 30 mg/kg/i.p./day for two weeks (standard drug).

Group 5: CCI + topiramate 20 mg/kg/i.p. /day for two weeks.

Group 6: CCI + topiramate 40 mg/kg/i.p. /day for two weeks.

Group 7: CCI + topiramate 80 mg/kg/i.p. /day for two weeks.

Induction of neuropathic pain by Streptozotocin (STZ)

The freshly prepared single STZ 60mg/kg solution was intraperitoneally administered according to body weight for the induction of diabetes.[18] After 72 hr of post-administration, the blood glucose level was measured using a glucometer strip (Nipro Diagnostic, India) for the confirmation of diabetes and rats with blood glucose level more than 250 mg/dl were used for further study.[22] These rats were included in the further study and the rest were discarded from the experimental protocol. The testing of behavioral parameters was done at the end of every week and a period of 28 days was taken for the development of neuropathic pain. On day 29th, the rats were treated with intraperitoneal topiramate at various doses like 20, 40, and 80 mg/kg body weight/day for the next two weeks.

In the present study, the six groups of rats were made each containing 06 rats (n = 06) as mentioned below.

Group 1: Normal control and received normal citrate buffer for two weeks.

Group 2: STZ treated rats with normal citrate buffer for two weeks.

Group 3: STZ treated rats received pregabalin 30 mg/kg/i/p//day for two weeks (standard drug).

Group 4: STZ treated rats received topiramate 20 mg/kg/i.p./day for two weeks.

Group 5: STZ treated rats received topiramate 40 mg/kg/i.p./day for two weeks.

Group 6: STZ treated rats received topiramate 80 mg/kg/i.p./day for two weeks.

Assessment of behavioral parameters

All the behavioral parameters were tested during the day portion of the circadian cycle i.e. between 09:00 am and 02:00 pm hr for two weeks post-induction of neuropathic pain.[23]

Mechanical allodynia by von Frey filaments

Initially, a force of 2.0 g was applied perpendicularly to the ipsilateral (left) hind paw until bending of filament (Aesthesio, Samitek Instruments, New Delhi) for 2 to 3 sec. If the rat fails to withdraw paw (negative response), the next filament with increased force (a stronger stimulus) was applied. If the rat withdraws paw or flinched paw (positive response), a filament with decreased weight (weaker stimulus) was applied for three times alternatively at an interval of 5 min between successive tests. The filament at which 50% of the application caused the paw withdrawal was considered as the paw withdrawal threshold (PWT) in grams (g).[19,23,24] An average of three readings was used for analysis.

Mechanical or static hyperalgesia by Randall-Selitto

The mechanical (static hyperalgesia) nociceptive threshold was evaluated by the pressure evoked stimulation method (Randall-Selitto Apparatus, Orchid scientific, Nashik). Removal of paw or vocalizations was considered as the endpoint and left hind paw was tested three times alternatively at an interval of 5 min between successive tests taking the cut-off force up to 450 g to avoid injury of the hind paw.[25] An average of three readings was used for analysis.

Mechanical or tactile hyperalgesia by pinprick

According to the method described by Bischofs, a modified pinprick test was used with a safety pin. The plantar surface of left hind paw used as application point where pin was applied perpendicularly (90° angles) and a cut-off time of 10 sec was considered to avoid possible tissue damage.[26] Each hind paw was tested thricely at an interval of 15 min between consecutive tests and the average of three readings was used for analysis.

Cold allodynia by acetone

The modified and standardized cotton bud method with a fixed volume of acetone (laboratory grade) cold allodynia was employed as described by Choi *et al.* (1984).[27] The plantar surface of left hind paw used as application point where stick was applied at an interval of 5–10 min thricely and 20 sec was considered as a cut-off time.[28-30] An average of three readings was used for analysis.

Heat hyperalgesia and heat allodynia by Eddy's hot plate

For the assessment of heat hyperalgesia and allodynia, the 45.5°C ± 0.5 and 52.5°C ± 0.5 were used in Eddy's hot plate (Orchid scientific, Nashik) respectively. Each hind paw was tested thricely at an interval of 15 min between consecutive tests to prevent sensitization of the hind paws.[31] An average of three readings was used for analysis.

Assessment of oxidative stress parameters

Tissue homogenization

At the end of the study, the blood sample was taken and centrifuged for estimation of glucose, and rats were sacrificed. After scarification, isolated sciatic nerve was kept in an ice-cold tris hydrochloric saline buffer of pH 7.4. The cross-chopped fine slices of tissue were suspended in chilled 0.25 M sucrose solution. After homogenization with 10% w/v of tris hydrochloric buffer (10 mM, pH7.4), it was subjected to cooling centrifugation at 10,000 rpm at 0°C for 15 min. The oxidative stress parameters, inflammatory markers, and total calcium were estimated in the clear supernatant.[32,33]

Estimation of lipid peroxide (MDA Content)

For estimation of lipid peroxidation, the method described by Slater and Sawyer was performed (1971). The assessment was carried out after the development of color at 532 nm against the reagent blank. The value was represented as nM of MDA/mg protein.[33]

Estimation of reduced Glutathione (GSH)

The tissue level of reduced Glutathione (GSH) homogenate was estimated by Moron (1979). The assessment was carried out after the development of color at 412 nm against the reagent blank. The value was represented as µg of GSH/mg protein.[34]

Estimation of Superoxide Dismutase (SOD)

The tissue activity for the SOD was performed by the Mishra method (1972). The changed optical density per min was measured after the addition of 0.4 mL of 3mM epinephrine at 480nm against blank and SOD activity was expressed as units/mg protein.[35]

Estimation of Catalase (CAT)

The tissue activity for the CAT was estimated by the method of Hugo Aebi method (1984).[36]

Estimation of Nitric Oxide (NO)

The tissue level of nitrite was estimated by the method of Guevara *et al.*, (1998).[37]

Estimation of total calcium

Total calcium level in the sciatic nerve was estimated by the method of Severinghaus and Ferrebee (1950) and modified by Muthuraman *et al.*[38] According to this method, the homogenate of the sciatic nerve was allowed to mix with ice-cold 1 mL of trichloroacetic acid (4%) followed by centrifugation under 2000 rpm for 10 m. The obtained supernatant was taken for the estimation of total calcium at 556 nm.

Assessment of inflammatory markers

The inflammatory markers such as TNF-α, IL-1β, and IL-6 in sciatic nerve supernatant were estimated using mouse TNF–α kit (eBioscience), a microtiter plate reader at 450 nm. Concentrations of TNF–α, IL-1β, and IL-6 were calculated from the standard curve.[39]

Histopathology of the sciatic nerve

After 28 days of surgery, the individual rat was sacrificed and the nerve was placed in 10% formalin solution for preservation. For the histopathological examination, the nerve was cut into the thickness of 4mm and further study staining was carried out in hematoxylin and eosin. For neuropathy, the section of the nerve was observed under the light microscope for overall histopathological changes.[40]

Statistical analysis

The Graph Pad Prism software version 5.0 was used for analysis of data. To determine the effect of treatment, results were analysed by one-way (ANOVA) followed by *post hoc* Dunnett's test. Data were represented as mean ± SEM. The level of statistical difference at $p<0.05$ was considered as significant.

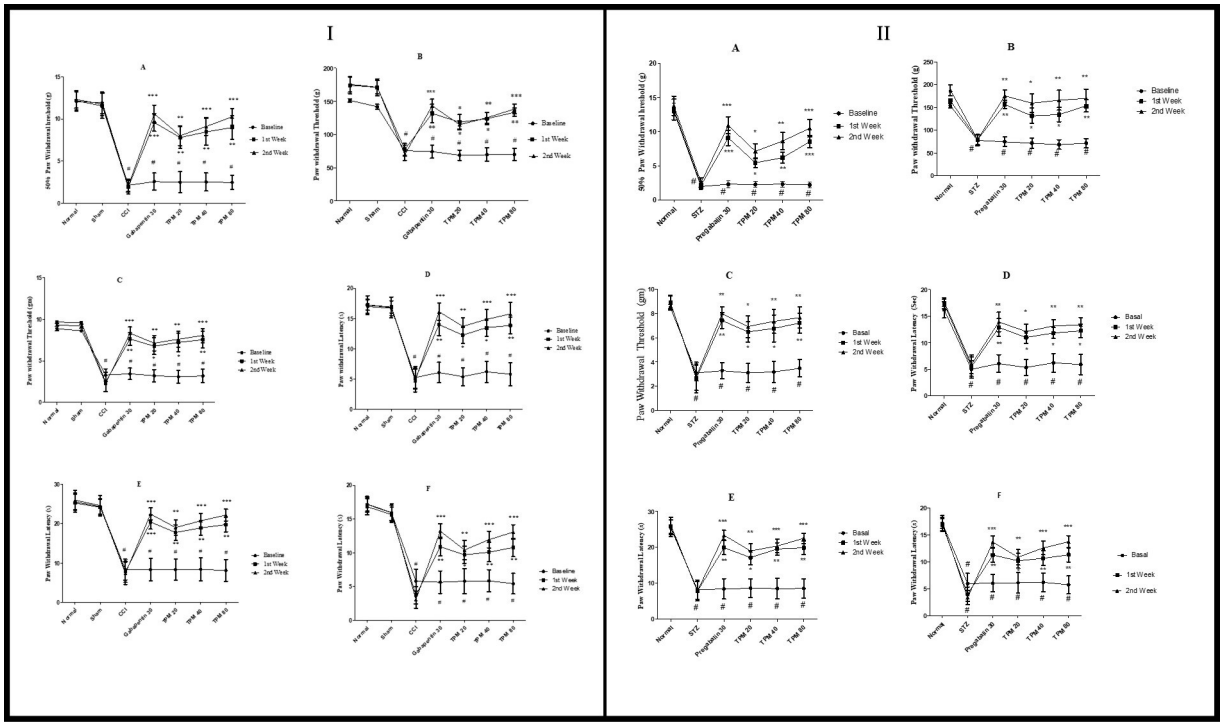

Figure 1: I. A) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical Allodynia tested by von Frey Filaments. B) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical or static hyperalgesia tested by Randall Sellitto Apparatus. C) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical or tactile hyperalgesia tested by pin prick. D) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Cold Allodynia tested by Acetone test. E) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Heat Allodynia tested Eddy's Hot Plate. F) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Heat Hyperalgesia tested Eddy's Hot Plate. Values expressed as mean ± S.E.M (n=10). Values are considered to be statistically significant at *$p<0.05$ vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

II. A) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical Allodynia tested by von Frey Filaments. B) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical or static hyperalgesia tested by Randall Sellitto Apparatus. C) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Mechanical or tactile hyperalgesia tested by pin prick. D) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Cold Allodynia tested by Acetone test. E) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Heat Allodynia tested Eddy's Hot Plate. F) The dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on Heat Hyperalgesia tested Eddy's Hot Plate. Values expressed as mean ± S.E.M (n=6). Values are considered to be statistically significant at *$p<0.05$ vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

RESULTS

Dose-dependent effect of topiramate on behavioral parameters in CCI and STZ induced neuropathy

Mechanical allodynia by von Frey filaments

The CCI to sciatic nerve significantly ($p<0.001$) decreases the paw withdrawal threshold as compared to the sham-operated group which was maintained till the end of the study period. There was no significant difference observed between the normal and sham-operated groups. The administration of topiramate 20, 40, and 80 mg/kg causes a dose-dependent increase in paw withdrawal threshold as compared to CCI rats which were evident from the first week of treatment ($p<0.01$, $p<0.01$, and $p<0.01$ respectively) and maintained till the end of the study period ($p<0.01$, $p<0.01$, and $p<0.001$ respectively). The gabapentin 30 mg/kg causes a significant ($p<0.001$) increase in the paw withdrawal threshold as compared to CCI rats throughout the treatment period as shown in Figure 1(IA).

The STZ-induced neuropathy was significantly ($p<0.001$) decreases the paw withdrawal threshold as compared to the normal group. The administration of topiramate 20, 40, and 80 mg/kg causes a dose-dependent increase in paw withdrawal threshold as compared to STZ rats which were evident from the first week of treatment ($p<0.05$, $p<0.01$, and $p<0.001$ respectively) and maintained till the end of the study period ($p<0.01$, $p<0.01$, and $p<0.001$ respectively). The pregabalin 30 mg/kg causes a significant ($p<0.001$) increase in the paw withdrawal threshold as compared to STZ rats as shown in Figure 1 (IIA).

Mechanical or static hyperalgesia by Randall-Selitto

After 14 days of treatment from the basal, the sciatic nerve ligation by CCI significantly ($p<0.001$) developed the mechanical hyperalgesia as compared to the sham-operated group and maintained throughout of the study period i.e. 28 days. After the first week of treatment with gabapentin, a significant ($p<0.01$) reversal of the development of mechanical hyperalgesia was observed as compared to CCI rats and maintained till the end of the study protocol ($p<0.001$). In the present study, it was observed that after the first week of treatment with different doses of topiramate like 20, 40, and 80 mg/kg significantly and dose-dependently increases the paw withdrawal threshold ($p<0.05$, $p<0.01$, and $p<0.01$ respectively) and, maintained for the second week also ($p<0.05$, $p<0.01$, and $p<0.001$ respectively) when compared with CCI rats as shown in Figure 1 (IB).

After 14 days of treatment from the basal, STZ-induced neuropathy significantly ($p<0.001$) developed the mechanical hyperalgesia as compared to the normal group. The treatment with pregabalin has significantly reversed the development of mechanical hyperalgesia as compared to STZ rats ($p<0.01$). The treatment with topiramate for 14 days significantly and dose-dependently increases the paw withdrawal threshold as compared to STZ rats ($p<0.05$, $p<0.05$, and $p<0.01$ respectively after the first week of treatment and ($p<0.05$, $p<0.01$, and $p<0.01$ respectively after the second week of treatment) as shown in Figure 1 (IIB).

Mechanical or tactile hyperalgesia by pinprick

After CCI to the sciatic nerve, the ipsilateral left hind paw became sensitive to mechanical tactile stimuli indicating the development of neuropathic pain states. The two weeks treatment with gabapentin significantly increases the paw withdrawal threshold ($p<0.01$ and, $p<0.001$ respectively) as compared to the CCI rats. The treatment with 20, 40, and 80 mg/kg topiramate for two weeks significantly and dose-dependently both delayed and attenuated the development of mechanical hyperalgesia ($p<0.05$, $p<0.05$, and $p<0.01$ for the first week and $p<0.01$, $p<0.01$, and $p<0.001$ for second week respectively) as shown in Figure 1 (IC).

After STZ administration, the ipsilateral left hind paw became sensitive to mechanical tactile stimuli which indicated the development of neuropathic pain. The treatment with pregabalin significantly increases the paw withdrawal threshold as compared to the STZ rats ($p<0.01$ for the first and second week). The treatment with 20, 40, and 80 mg/kg topiramate for two weeks significantly and dose-dependently both delayed and attenuated the development of mechanical hyperalgesia ($p<0.05$, $p<0.05$, and $p<0.01$ for the first week and $p<0.01$, $p<0.01$, and $p<0.01$ for second week respectively) as shown in Figure 1 (IIC).

Cold allodynia by acetone

The CCI to sciatic nerve to left hind paw causes a significant ($p<0.001$) reduction in the paw withdrawal latency as compared to the sham-operated group. The treatment with gabapentin significantly increases the paw withdrawal latency as compared to the CCI rats ($p<0.01$ for the first week and $p<0.001$ for the second week respectively). Following administration of topiramate with different doses like 20, 40, and 80 mg/kg after the first week produces a significant increase in paw withdrawal latency ($p<0.05$, $p<0.05$, and $p<0.01$ respectively) and, this effect was maintained for the second week also ($p<0.01$, $p<0.001$, and $p<0.001$ respectively) when compared with CCI rats as shown in Figure 1 (ID).

The STZ induced neuropathy causes a significant reduction in the left paw withdrawal latency as compared to the normal group ($p<0.001$). The treatment with pregabalin significantly increases the paw withdrawal latency as compared to the STZ rats ($p<0.01$ for two weeks). Following administration of topiramate with different doses like 20, 40, and 80 mg/kg after the first week produces a significant increase in paw withdrawal latency ($p<0.05$, $p<0.05$, and $p<0.05$ respectively) and, this effect was maintained for the second week also ($p<0.05$, $p<0.01$, and $p<0.01$ respectively) when compared with STZ rats as shown in Figure 1 (IID).

Heat hyperalgesia and heat allodynia by Eddy's hot plate

In the present study, it was observed that the CCI to left sciatic nerve causes significant (p<0.001) reduction in paw withdrawal latency in both heat hyperalgesia test (52.5 ± 0.5°C) and heat allodynic test (45 ± 0.5°C) as compared to the sham-operated group. The gabapentin reversed the development of heat hyperalgesia and allodynia and significantly increases the paw withdrawal latency as compared to CCI rats (p<0.01, and p<0.001 for heat allodynia and p<0.001 for heat hyperalgesia). The present study protocol revealed that ingestion of different concentrations of topiramate 20, 40, and 80 mg/kg significantly and dose-dependently (p<0.01, and p<0.001 respectively) attenuated the development of heat allodynia throughout the study period. Furthermore, it also prevented the development of hyperalgesia throughout the study period (p<0.01, and p<0.001 respectively). Further, this study protocol revealed that topiramate significantly increases the paw withdrawal latency as compared to CCI rats as shown in Figure 1 (IE and IF respectively).

In the present study, it was observed that the STZ-induced neuropathy causes a significant (p<0.001) reduction in left paw withdrawal latency in both heat hyperalgesia test (52.5 ± 0.5°C) and heat allodynic test (45 ± 0.5°C) as compared to the normal group. The pregabalin reversed the development of heat hyperalgesia and allodynia and significantly increases the paw withdrawal latency as compared to STZ rats (p<0.01, and p<0.001 for heat allodynia and hyperalgesia). The present study protocol revealed that ingestion of different concentrations of topiramate 20, 40, and 80 mg/kg significantly and dose-dependently (p<0.05, p<0.01, and p<0.01 for the first week and p<0.01, p<0.001, and p<0.001 for second week respectively) attenuated the development of heat allodynia throughout the study period. Furthermore, it also prevented the development of hyperalgesia throughout the study period (p<0.05, p<0.01, and p<0.01 for the first week and p<0.01, p<0.001, and p<0.001 for the second week respectively). Further, this study protocol revealed that topiramate significantly increases the paw withdrawal latency as compared to STZ rats as shown in Figure 1 (IIE and IIF respectively).

Dose-dependent effect of topiramate on oxidative stress parameters in CCI and STZ induced neuropathy

Estimation of lipid peroxide (MDA Content)

The CCI to the sciatic nerve causes a significant increase (717.6 ± 59.33, p<0.001) in the MDA as compared to sham-operated rats (208.8 ± 37.90). The MDA level in gabapentin-treated rats significantly (247.6 ± 46.29, p<0.001) decreased as compared to CCI rats. Likewise, attenuation of increased MDA level by different doses of topiramate 20, 40, and 80mg/kg treatment (480.4 ± 83.22 and p<0.05, 400.6 ± 62.60 and p<0.01 and 323.5 ± 60.14 and p<0.001 respectively) was more significant and

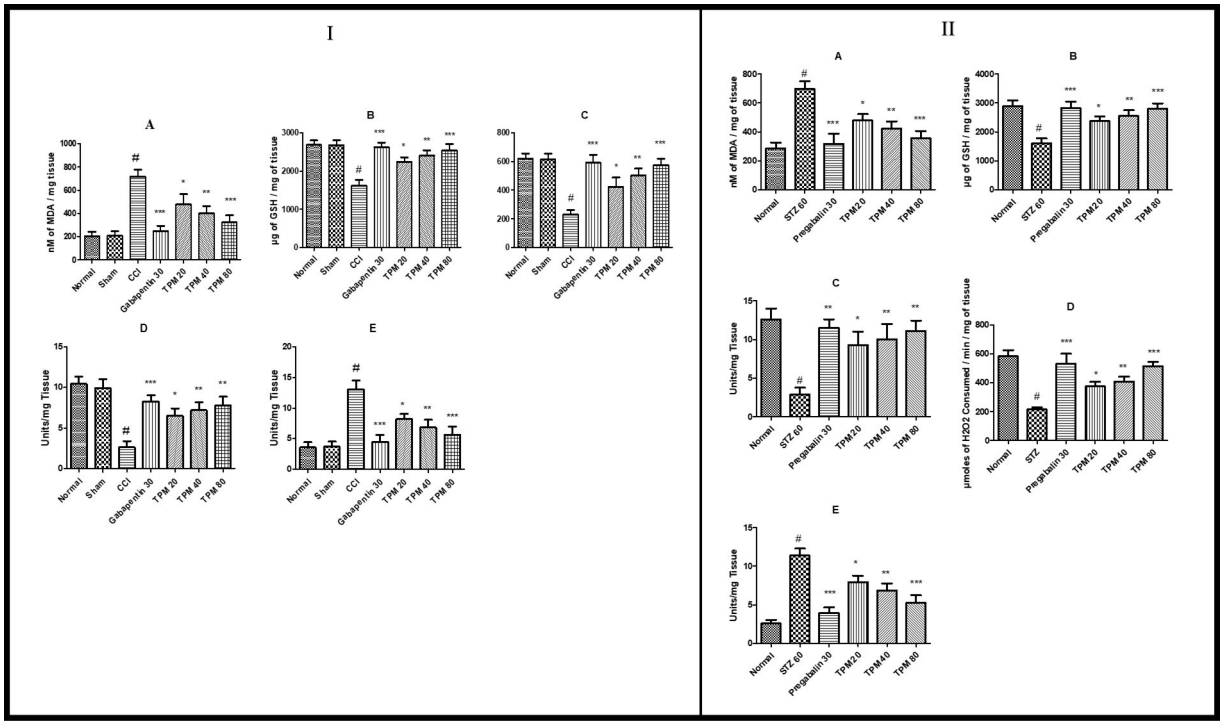

Figure 2: I. Dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on MDA (A), GSH (B), CAT (C), SOD (D), and NO (E). Values expressed as mean ± S.E.M (n=10). Values are considered to be statistically significant at *p<0.05 vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

II. Dose-dependent effect of Topiramate (20, 40 and 80 mg/kg) on LPO or MDA (A), GSH (B), SOD (C), CAT (D), and NO (E). Values expressed as mean ± S.E.M (n=6). Values are considered to be statistically significant at *p<0.05 vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

dose-dependent as compared to CCI rats as shown in Figure 2(IA).

The STZ induced neuropathy causes a significant increase (698.7 ± 51.93, $p<0.001$) in the MDA level as compared to normal rats (285.4 ± 40.05). The MDA level in pregabalin-treated rats significantly (317.4 ± 69.37, $p<0.001$) decreased as compared to STZ rats. Likewise, attenuation of increased LPO level by topiramate treatment (478.9 ± 43.98 and $p<0.05$, 421.7 ± 49.77 and $p<0.01$ and 356.7 ± 49.36 and $p<0.001$ respectively) was more significant and dose-dependent as compared to STZ rats as shown in Figure 2 (IIA).

Estimation of reduced Glutathione (GSH)

The GSH level in CCI-treated rats was significantly (1617. ± 149.3, $p<0.001$) decreased as compared to sham-operated rats (2680. ± 127.7). The two weeks treatment of topiramate significantly and dose-dependently (2239 ± 123.2 and $p<0.05$, 2405 ± 141.7 and $p<0.01$ and 2545 ± 164.4 and $p<0.001$ respectively) elevated the level of GSH as compared to CCI rats. The GSH level in standard gabapentin-treated rats (2623 ± 115.2, $p<0.001$) was significantly increased as compared to CCI rats as shown in Figure 2 (IB).

The GSH level in STZ treated rats was significantly (1600 ± 181.5, $p<0.001$) decreased as compared to normal rats (2888 ± 195.7). The two weeks treatment of topiramate significantly and dose-dependently (2372 ± 169.3 and $p<0.05$, 2553 ± 195.2 and $p<0.01$ and 2800 ± 183.3 and $p<0.001$ respectively) elevated the level of GSH as compared to STZ rats. The GSH level in standard pregabalin-treated rats (2822 ± 222.3, $p<0.001$) was significantly increased as compared to STZ rats as shown in Figure 2 (IIB).

Estimation of Catalase (CAT)

The two weeks of post-surgery of the sciatic nerve by CCI was significant (228.1 ± 34.48 and $p<0.001$) resulted in a decrease in the level of CAT as compared to sham-operated rats (616.1 ± 38.50). In the present study, it was observed that the treatment of topiramate dose-dependently and significantly (423.1 ± 65.06 and $p<0.05$, 500.8 ± 48.86 and $p<0.01$ and 574.5 ± 44.52 and $p<0.01$ respectively) prevented the decrease in the level of CAT as compared to CCI rats. Moreover, the treatment of gabapentin significantly (590.7 ± 55.64 and $p<0.01$) reversed the decrease in the CAT level as compared to CCI rats as shown in Figure 2 (IC).

The STZ induced neuropathy was significant (214.0 ± 16.04 and $p<0.001$) resulted in a decrease in the level of CAT as compared to normal rats (583.2 ± 41.56). In the present study, it was observed that the treatment of topiramate dose-dependently and significantly (374.5 ± 31.54 and $p<0.05$, 408.0 ± 32.17 and $p<0.01$ and 513.6 ± 30.81 and $p<0.01$ respectively) prevented the decrease in the level of CAT as compared to STZ rats. Moreover, the treatment of pregabalin significantly (528.7 ± 70.55 and $p<0.01$) reversed the decrease in the CAT level as compared to STZ rats as shown in Figure 2 (IIC).

Estimation of Superoxide Dismutase (SOD)

The two weeks after CCI to sciatic nerve was associated with a significantly (2.645 ± 0.7671 and $p<0.001$) decreased level of SOD as compared to sham-operated rats (9.943 ± 1.068). The decreased level of SOD condition was significant and dose-dependently (6.505 ± 0.9267 and $p<0.05$, 7.203 ± 0.9995 and $p<0.01$ and 7.805 ± 1.062 and $p<0.01$ respectively) reversed by the two weeks' treatment with topiramate as compared to CCI rats. The decreased level of SOD significantly (8.305 ± 0.7523 and $p<0.001$) was brought to the normal level by the treatment with gabapentin as compared to CCI rats as shown in Figure 2 (ID).

The STZ induced neuropathy was associated with a significantly (2.885 ± 0.8675 and $p<0.001$) decreased level of SOD as compared to normal rats (12.62 ± 1.410). The decreased level of SOD condition was significant and dose-dependently (9.302 ± 1.739 and $p<0.05$, 10.03 ± 1.998 and $p<0.01$ and 11.14 ± 1.321 and $p<0.01$ respectively) reversed by the two weeks' treatment with topiramate as compared to STZ rats. The decreased level of SOD significantly (11.50 ± 1.128 and $p<0.001$) was brought to the normal level by the treatment with pregabalin as compared to STZ rats as shown in Figure 2 (IID).

Estimation of Nitric Oxide (NO)

A significant (13.06 ± 1.184 and $p<0.001$) increase in the nitrite level was observed in CCI rats as compared to sham-operated rats (3.767 ± 0.777). The topiramate produced a dose-dependent (8.183 ± 0.8448 and $p<0.05$, 6.833 ± 1.256 and $p<0.01$ and 5.667 ± 1.302 and $p<0.01$ respectively) decrease in nitrite levels, where rats were treated with topiramate showed significantly lower levels of nitrite as compared to CCI rats. The rats treated with gabapentin significantly (4.504 ± 1.129 and $p<0.001$) attenuated the increased level of nitrite as compared to CCI rats as shown in Figure 2 (IE).

A significant 11.38 ± 0.9327 and $p<0.001$) increase in the nitrite level was observed in STZ treated rats (as compared to normal rats (2.585 ± 0.4760). The topiramate produced a dose-dependent decrease in nitrite levels, where rats were treated with topiramate showed significantly (7.950 ± 0.8391 and $p<0.05$, 6.875 ± 0.8934 and $p<0.01$ and 5.252 ± 1.007 and $p<0.01$ respectively) lower levels of nitrite as compared to STZ rats. The rats treated with pregabalin significantly (3.913 ± 0.7689 and $p<0.001$) attenuated the increased level of nitrite as compared to STZ rats as shown in Figure 2 (IIE).

Dose-dependent effect of topiramate on inflammatory markers and total calcium in CCI and STZ induced neuropathy

TNF-α measurement

The CCI to sciatic nerve resulted in a significant (2212 ± 212.6 and $p<0.001$) increase in the level of TNF-α as compared

to sham-operated rats (724.7 ± 249.9). The present finding showed that administration of topiramate significantly and dose-dependently (1302 ± 196.4 and $p<0.05$, 1094 ± 197.8 and $p<0.01$ and 882.8 ± 188.9 and $p<0.001$) alleviated the increased level of TNF-α level. The rats treated with gabapentin significantly alleviated the increased level of TNF-α as compared to CCI rats (785.7 ± 223.0 and $p<0.001$as shown in Figure 3(IA).

The STZ induced neuropathy resulted in a significant (2324 ± 207.0 and $p<0.001$) increase in the level of TNF-α as compared to normal rats (770 ± 205.4). The present finding showed that administration of topiramate significantly and dose-dependently (1444 ± 217.5 and $p<0.05$, 1153 ± 200.4 and $p<0.01$ and 921.7 ± 193.6 and $p<0.001$) alleviated the increased level of TNF-α level. The rats treated with pregabalin significantly alleviated the increased level of TNF-α as compared to STZ rats (867.5 ± 227.2 and $p<0.001$as shown in Figure 3 (IIA).

IL–1beta and IL-6 measurement

The CCI to sciatic nerve resulted in a significant (1543 ± 187.5 and $p<0.001$) increase in the level of IL–1 beta as compared to sham-operated rats (365.8 ± 130.1). The present finding showed that administration of topiramate significantly and dose-dependently (719.3 ± 175.8 and $p<0.01$, 601.3 ± 182.9 and $p<0.01$ and 504.2 ± 154.0 and $p<0.001$) alleviated the increased level of IL–1 beta level. The rats treated with gabapentin significantly alleviated the increased level of IL–1 beta as compared to CCI rats (468.7 ± 147.3 and $p<0.001$as shown in Figure (IB). The CCI to sciatic nerve resulted in a significant (1667 ± 190.4 and $p<0.001$) increase in the level of IL–6 as compared to sham-operated rats (330.7 ± 120.2). The present finding showed that administration of topiramate significantly and dose-dependently (804.5 ± 199.3 and $p<0.05$, 591.3 ± 195.8 and $p<0.01$ and 460.3 ± 203.2 and $p<0.001$) alleviated the increased level of IL–6 level. The rats treated with gabapentin significantly alleviated the increased level of IL – 6 as compared to CCI rats (404.7 ± 201.9 and $p<0.001$as shown in Figure 3 (IC).

The STZ induced neuropathy resulted in a significant (1599 ± 193.6 and $p<0.001$) increase in the level of IL–1 beta as compared to normal rats (384.8 ± 131.8). The present finding showed that administration of topiramate significantly and dose-dependently (819 ± 170.3 and $p<0.01$, 662.8 ± 183.6 and $p<0.01$ and 549.2 ± 163.2 and $p<0.001$) alleviated the increased level of IL–1 beta level. The rats treated with pregabalin significantly alleviated the increased level of IL–1 beta as compared to STZ rats (496.5 ± 155.3 and $p<0.001$as shown in Figure (IB). The STZ induced neuropathy resulted in a significant (1748 ± 192.6 and $p<0.001$) increase in the level of IL–6 as compared to normal rats (373.7 ± 136.9). The present finding showed that administration of topiramate significantly and dose-dependently (847.7 ± 210.1 and $p<0.05$, 613.3 ± 207.5 and $p<0.01$ and 494.8 ± 192.9 and $p<0.001$)

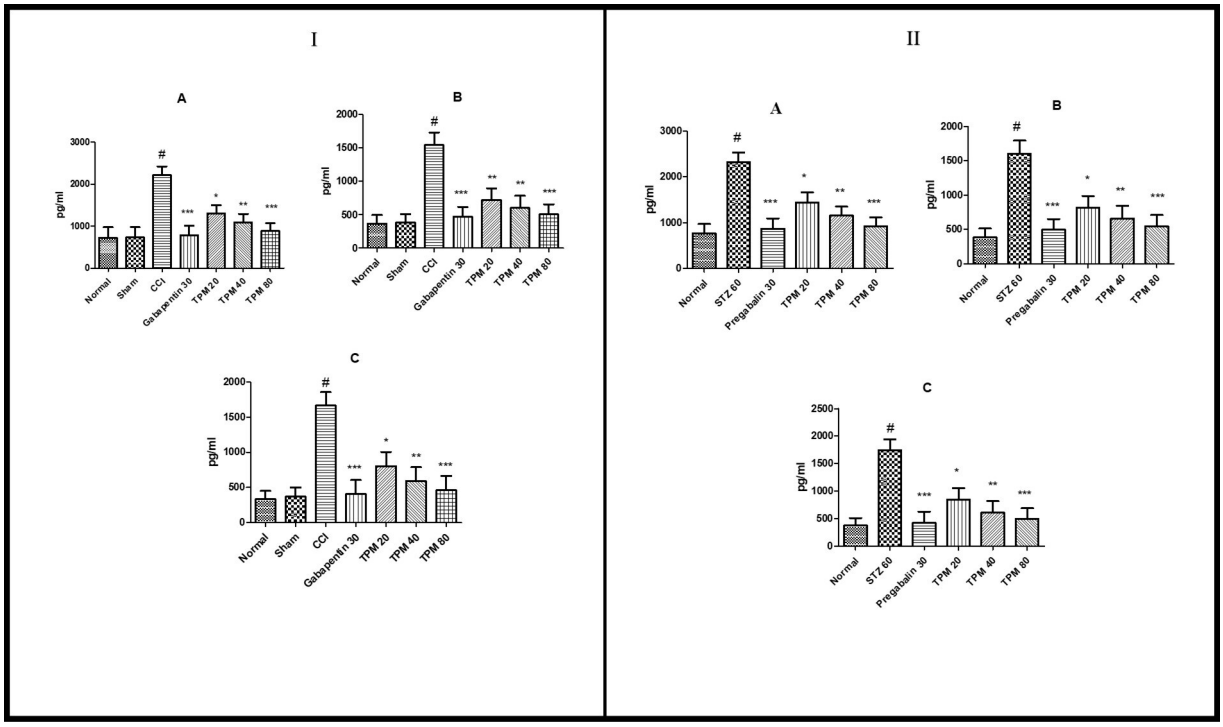

Figure 3: I. A) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on TNF-alpha. B) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on IL-1beta. C) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on IL-6. Values expressed as mean ± S.E.M (n=10). Values are considered to be statistically significant at *$p<0.05$ vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

II. A) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on TNF-alpha. B) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on IL-1beta. C) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on IL-6. Values expressed as mean ± S.E.M (n=6). Values are considered to be statistically significant at *$p<0.05$ vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

alleviated the increased level of IL–6 level. The rats treated with pregabalin significantly alleviated the increased level of IL–6 as compared to STZ rats (424.4 ± 204.6 and $p<0.001$as shown in Figure 3 (IIC).

Effect on total calcium

Sciatic nerve injury caused by CCI significantly (19.77 ± 2.091 and $p<0.001$) increases the tissue level of total calcium as compared to the sham-operated group. Administration of topiramate significantly (12.42 ± 1.181 and $p<0.05$, 10.88 ± 1.125 and $p<0.01$, 7.720 ± 1.679 and $p<0.001$) reduces the tissue level of calcium as compared to the CCI group in a dose-dependent manner. Treatment of gabapentin also reduces the increased level of tissue calcium level as compared to the CCI group (6.230 ± 1.595 and $p<0.001$) as shown in Figure 4(A).

Similarly, administration of STZ causes an increase (22.16 ± 2.565 and $p<0.001$) in the level of total calcium in sciatic nerve homogenate as compared to the normal group. Administration of topiramate significantly (13.82 ± 1.299 and $p<0.05$, 12.26 ± 1.182 and $p<0.01$, 9.760 ± 1.737 and $p<0.001$) reduces the tissue level of calcium as compared to the STZ group in a dose-dependent manner. Treatment of gabapentin also reduces the increased level of tissue calcium level as compared to the STZ group (7.940 ± 1.568 and $p<0.001$) as shown in Figure 4 (B).

Effect of topiramate on serum blood glucose in STZ neuropathy

Following STZ administration, the serum glucose was estimated to confirm the state of hyperglycemia. Serum fasting glucose levels were estimated on Day 31 (basal), Day 38 (posttreatment 1st week), and Day 45 (post-treatment 2nd week). Intraperitoneal administration of topiramate at three different dose levels of 20, 40, and 80 mg/kg has non significantly reduced serum glucose levels throughout the treatment period as shown in Figure 4 (C).

Histopathological Examination

In the present CCI-induced and STZ induced neuropathic pain study, it was observed that the sciatic nerve was significantly subjected to myelin sheath swelling, degeneration, and necrosis. There were no changes were observed in normal and sham-operated groups. The treatment with topiramate dose-dependently and significantly prevented both CCI and STZ-induced sciatic nerve damage (supplementary file) and Tables 1 and 2 respectively.

DISCUSSION

Numerous current works highlighted the severe consequences of neuropathy on health of an individuals which adversely affects physical, emotional, and social wellbeing. While addressing the problem of neuropathy, the treatment must be targeted not only at general symptoms, intensity of pain, and its severeness like acuteness or chronicity, but also at the underlying neurobiological mechanisms and molecular targets. Further, there is a need to

Table 1: Effect of Topiramate on Histopathological Examination of Sciatic Nerve following CCI.

Groups	Swelling in Sheath	Degeneration	Necrotic Changes
Normal	-	-	-
Sham	+	+	+
CCI	+++	+++	+++
Gabapentin 30	+	+	+
TPM 20	++	+	++
TPM 40	++	+	+
TPM 80	+	+	+

(Where, +++ - Severe; ++ - Moderate; + - Mild; - Nil)

Table 2: Effect of Topiramate on Histopathological Examination of Sciatic Nerve after STZ.

Groups	Swelling in Sheath	Degeneration	Necrotic Changes
Normal	-	-	-
STZ	+++	+++	+++
Pregabalin 30	+	+	+
TPM 20	++	++	+
TPM 40	++	+	+
TPM 80	+	+	+

(Where, +++ - Severe; ++ - Moderate; + - Mild; - Nil)

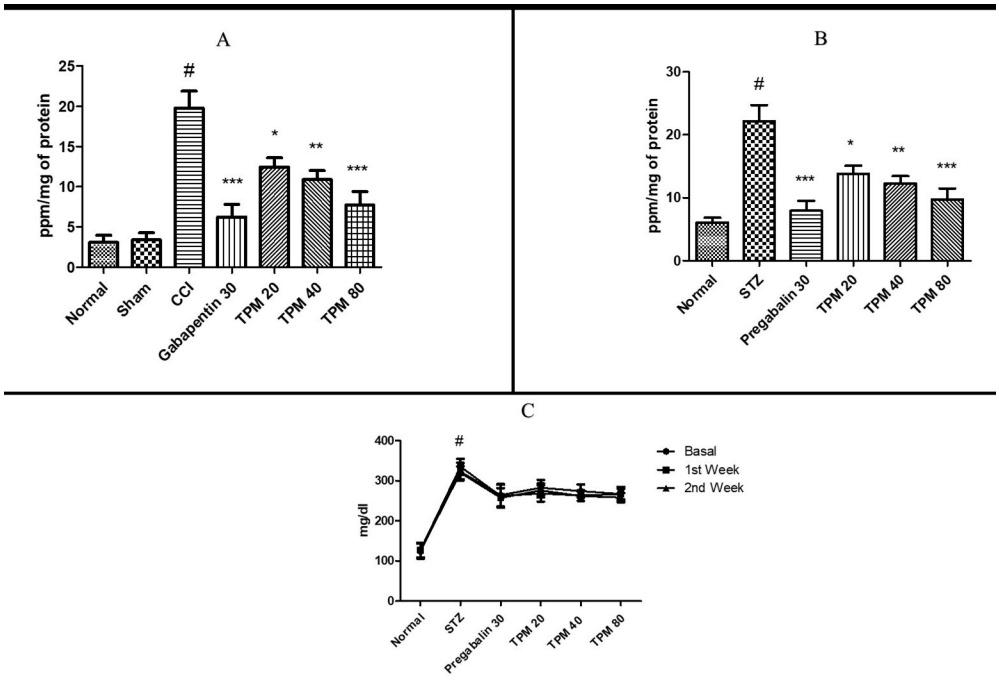

Figure 4: A) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on total calcium level. Values expressed as mean ± S.E.M (*n*=10). Values are considered to be statistically significant at **p*<0.05 vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test). B) Dose dependent effect of topiramate (20, 40 and 80 mg/kg) on total calcium level. Values expressed as mean ± S.E.M (*n*=6). Values are considered to be statistically significant at **p*<0.05 vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test). C) Effect of topiramate (20, 40 and 80 mg/kg) on serum blood glucose. Values expressed as mean ± S.E.M (*n*=10). Values are considered to be statistically significant at **p*<0.05 vs. normal group (One-Way ANOVA followed by Dunnett *Post hoc* test).

develop the efficient therapeutic agents that specifically disturb the specific pain mechanisms.[41]

Physicians routinely prescribed the anticonvulsant agents in the management of neuropathy based on their experience with neuropathy. Firstly, these agents are enthusiastically used in neuropathy but care has been taken in dealing with other types of pain. Despite this, their use as an analgesic in neuropathy still remain unclear.[42] Fundamentally, the pathogenesis of seizure and neuropathic pain follows similar pattern of neuronal hyperexcitability and several clinicians and researchers believed that anticonvulsants are best therapeutic options for neuropathy.[43] The substantial empirical data for various anticonvulsants like phenytoin, valproic acid, carbamazepine, gabapentin, lamotrigine, oxcarbazepine, zonisamide, levetiracetam, tiagabine, and topiramate for their effectiveness in management of neuropathy is available. Among the several tested agents, very few of them produced favourable actions, and clinically, gabapentin is an approved first choice of drug for the successful treatment of various types of neuropathies.[44]

Chemically, topiramate is a 2,3,4,5-bis-o-[1-methyethylidene]-36-D-fructopyramose sulfamate derivative with inhibitory effects on carbonic anhydrase enzyme. Clinically, it is approved for the management of various disorders like simple or complex partial seizure, primary generalized seizure and Lennox-Gastaut syndrome.[13] Electrophysiological and biochemical empirical data of topiramate reveal its mechanism of action which is characterized by blockade of voltage-dependent Na^+ and Ca^{2+} channel, enhancement of GABA and GABAergic activity, inhibition of AMPA glutamate receptors, and antioxidant property.[14,15] In the present study, we have explored the dose-dependent neuroprotective effects of intraperitoneal administration of topiramate at a dose of 20, 40, and 80mg/kg body weight for two weeks employing Chronic Constriction Injury (CCI) and Streptozotocin (STZ) preclinical models.

Lesion to the peripheral nervous system may leads to transformation into severe neuropathic pain condition. Experimentally, this chronic pain condition is well established by using CCI to sciatic nerve and STZ animal model of peripheral nerve injury.[45,46] Again, these models are associated with development of characteristic symptoms of neuropathy like allodynia and hyperalgesia. The present study showed that CCI and STZ lead to elevated levels of proinflammatory cytokines like TNF-α, IL-1β, and IL-6 and total calcium in homogenate of sciatic nerve. These modulatory changes cause neuroinflammation, damage of myelin sheath and Wallerian degeneration of peripheral nerve. Furthermore, these changes lead to formation

of Reactive Oxygen Species (ROS) that transform into the burden of oxidative stress. Formed ROS shifted the normal antioxidant defence mechanism to oxidative mechanism by causing increased state of lipid peroxidation, decreased Glutathione Peroxidase (GSH), increased nitric oxide synthesis, and minimizes the level of catalase and superoxide dismutase. Togetherly, increased level of inflammatory cytokines and oxidative stress burden initiates and progress the state of exaggerated response of both peripheral and central neurons that leads to development of characteristic symptoms of neuropathy like allodynia and hyperalgesia.[30,47]

In the current study, CCI and STZ decrease paw withdrawal threshold in pin-prick and Randall-Sellitto tests[28,48] and von Frey.[49,50] It also decreases paw withdrawal latency in both Eddy's hot plate induced heat allodynia and hyperalgesia and acetone induced cold allodynia.[51] Our result findings are in line with previous experiments where the CCI and STZ associated with decrease in paw withdrawal threshold and latency.[23,50,52-55] In the present experiment we provided new insights that the post-surgery intraperitoneal administration of topiramate for two weeks at a dose of 20, 40, and 80 mg/kg body weight significantly and dose-dependently delayed and prevented the development of allodynia and hyperalgesia. Based on experiments carried by Benoliel and his co-workers, it was observed that topiramate at a dose of 20mg/kg/day for 13 days produces analgesic effect as it significantly postponed onset and prevented the development of allodynia as well as hyperalgesia.[56] Also, analgesic potential of topiramate was successfully tested with well standardized preclinical models of neuropathy such as partial peripheral nerve injury, spinal nerve ligation, nerve crush model and CCI.[56,57]

Being rich in lipids, the peripheral nervous system is vulnerable to deleterious effects of lipid peroxidation and hydrogen peroxide. In this context, the various antioxidant parameters such as Superoxide Dismutase (SOD), Catalase (CAT), and GPx play a protective and defensive role against oxidative stress.[48,58] The previous reports with CCI and STZ showed that the concentration of Xanthine Oxidase (XO), inducible Nitric Oxide Synthase (iNOS) in macrophages and Schwann cells and NO were elevated.[59] Also, extensive research was carried out to explore mechanism of CCI and STZ induced oxidative stress and alterations in antioxidative defence system in neuropathy. In the present study, it was found that the CCI and STZ causes the development of oxidative stress by elevating the sciatic nerve tissue level of various endogenous enzymes such as Lipid Peroxide (LPO) and Nitric Oxide Synthase (NOS) as well as decreased the level of GSH, SOD, and CAT.[60,61] The two weeks treatment of topiramate dose-dependently and significantly prevented the development of oxidative stress by elevating the level of GSH, SOD and CAT, and decreasing the level of LPO and NOS. The similar experiments were carried out in this direction to highlight the neuroprotective and antioxidants property of topiramate in cerebral ischemia and epilepsy experimental models where it causes an increase in GSH, SOD and CAT level, and decreases LPO and NOS levels.[26,62] Also, topiramate prevented the development of lipid peroxidation by increasing the brain GSH level in epileptic mice.

The extensive literature with CCI and STZ showed that the injury to sciatic nerve was associated with alterations in the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and total calcium. The CCI and STZ causes significant elevation of the levels of TNF-α, IL-1beta, and IL–6 in the sciatic nerve which was associated with the development of hyperalgesia.[54,59] In the present experiment, the treatment with topiramate produced a significant and dose-dependent decrease in the level of TNF-α, IL-1beta, and IL–6. It has been observed that both clinical and preclinical experimental model of epilepsy was associated with elevated expression of endogenous pro-inflammatory cytokines. These elevated cytokines are responsible for the development of seizures particularly status epilepticus.[59] Also, topiramate administration exerts modulatory effect on expression of anti-inflammatory cytokines such as IL-10 which is linked with its antiseizure effects in kainite-injected rats.

The histopathological examination showed that CCI and STZ induced neuropathies were associated with swelling in myelin sheath, sciatic nerve degeneration and necrosis.[40] The treatment with topiramate dose-dependently and significantly attenuated the histological abnormalities in the sciatic nerve.

CONCLUSION

Finally, the current study found that CCI and STZ-induced peripheral neuropathy were associated with oxidative stress and increased cytokine levels. These modifications alter the neuronal plasticity that results in hyperalgesia and allodynia. Topiramate doses of 20, 40, and 80 mg/kg significantly and dose-dependently reduced the levels of oxidative markers and cytokines, preventing the typical symptoms of neuropathy such as hyperalgesia and allodynia. These findings suggest that topiramate may have analgesic, antioxidant, and anti-inflammatory effects in the treatment of neuropathy in a dose-dependent manner.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TNF-α: Tumor necrosis factor-α**; IL-β:** Interleukin-β; **ROS:** Reactive oxygen species; **AMPA:** α-amino-3-hydroxy-5methyl-4-isoxazolepropinoic acid; **GABA:** γ-amino butyric acid; **NO:** Nitric oxide; **MDA:** Malonaldehyde; **GSH:** Reduced glutathione; **SOD:** Superoxide dismutase; **CAT:** Catalase; **CCI:** Chronic constriction injury; **STZ:** Streptozotocin.

SUMMARY

In most condition, the symptomatic relief from pain is achieved after taking relevant medications, although the other complications like diabetes mellitus and peripheral nerve injury that cannot completely cured and the management of pain in these conditions become prime and critical goal. The multi-functional and mechanistic quality of topiramate provides a promising therapeutic approach for the management of neuropathy. Besides, its broad spectrum and novel anticonvulsant property, topiramate produces antagonistic effects on glutamate receptors, modulates the effects of voltage gated sodium and calcium channel activity, and accelerates the GABA mediated inhibitory effects. In the current experiments, we provide main evidence proof in the context of dose dependent neuroprotective effect of topiramate following CCI and STZ. Topiramate attenuated the CCI and STZ induced neuronal, biochemical, and behavioral changes in the sciatic nerve. These neuroprotective effects are possibly responsible for analgesic, antioxidant and anti-inflammatory effects (Supplementary File).

Author Contribution

Conceptualization: A.B.U.; C.D.U.; **Methodology:** A.K.S.; A.B.U.; C.D.U.; **Investigation:** A.K.S.; C.D.U.; **Formal Analysis:** A.K.S.; A.B.U.; **First Draft of Manuscript:** A.K.S.; A.B.U.; **Manuscript Editing:** A.B.U.; C.D.U.

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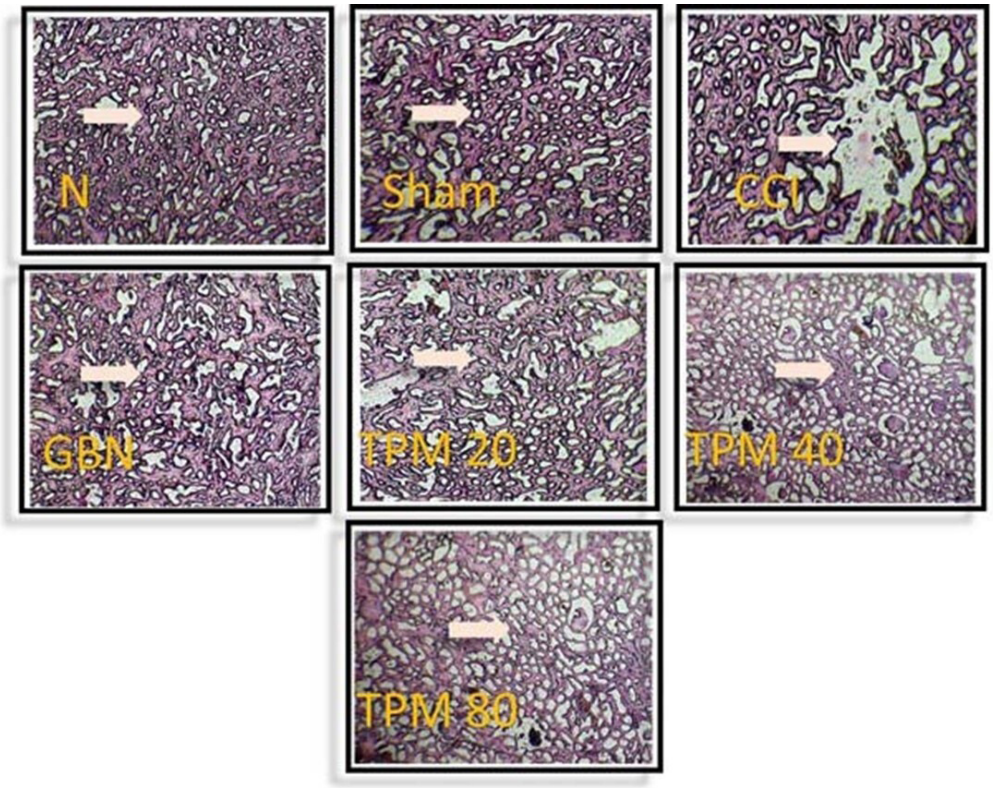

Figure S1: Effect of Topiramate on Histopathology of Sciatic Nerve. The normal and sham transverse section of Sciatic shown normal structure. The section of CCI showed sciatic nerve myelin sheath swelling, degeneration and necrosis. The effect of gabapentin 30 mg/kg attenuated sciatic nerve myelin sheath swelling, degeneration and necrosis due to CCI. The effect of topiramate (20, 40 and 80 mg/kg) dose dependently and significantly attenuated the sciatic nerve myelin sheath swelling, degeneration and necrosis due to CCI respectively.

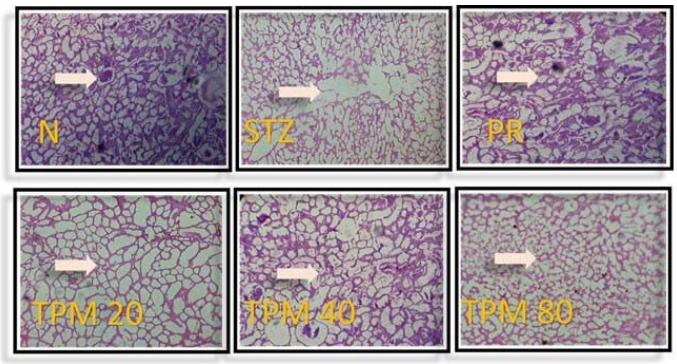

Figure S2: Effect of Topiramate on Histopathology of Sciatic Nerve. The normal and sham transverse section of Sciatic shown normal structure. The section of CCI showed sciatic nerve myelin sheath swelling, degeneration and necrosis. The effect of gabapentin 30mg/kg attenuated sciatic nerve myelin sheath swelling, degeneration and necrosis due to CCI. The effect of topiramate (20, 40 and 80mg/kg) dose dependently and significantly attenuated the sciatic nerve myelin sheath swelling, degeneration and necrosis due to CCI respectively.

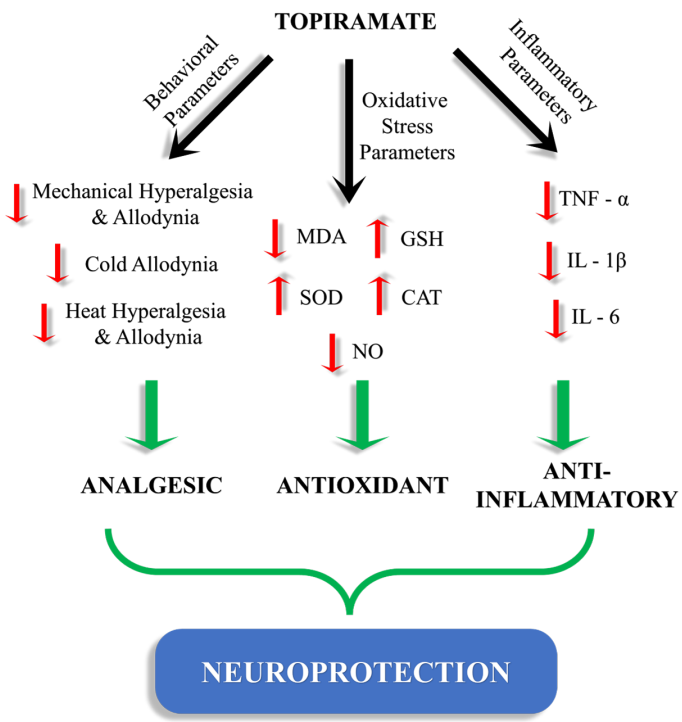

Figure S3: Neuroprotective Effects of Topiramate in CCI and STZ Induced Neuropathy.