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Article

Alleviation of neuropathic pain by trazodone in rats

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Neuropathic pain is generally characterised by an abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia). The present study was designed to investigate the effect of trazodone (5mg/kg and 10mg/kg) on peripheral neuropathic pain induced by partial sciatic nerve ligation in rats. Mechanical hyperalgesia, cold allodynia and thermal hyperalgesia were assessed by performing the pinprick, acetone, and hot plate tests, respectively. Biochemically, lipid peroxidation level and total calcium levels were measured. However, trazodone administration (5 and 10 mg/kg i.p.) for 21days significantly diminished partial sciatic nerve ligation-induced neuropathic pain along with areduction in oxidative stress and calcium levels. The results of the present study suggest that trazodone is effective in attenuating partial sciatic nerve ligation-inducedpainful neuropathic states, which may be attributed to decreased oxidative stress and calcium levels.

Keywords: Hyperalgesia. Cold allodynia. Partial sciatic nerve ligation. Trazodone.

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INTRODUCTION

Neuropathic pain is usually chronic andcaused by damage or disease affecting the somatosensory system. Neuropathic pain may be associated with abnormal sensations called dysesthesias (pain that occurs spontaneously), allodynia (pain as a result of stimulus thatdoes not normally provoke pain) or hyperalgesia (an increased response to a stimulus thatis normally painful). In this complex syndrome, some maladaptive variations are found in the entire nociceptive pathway within the central nervous system.

Neuropathic pain (Treede *et al*., 2008) may result from disorders of the peripheral nervous system or the central nervous system (brain and spinal cord). Up to 7-8% of the global human population is affected, and it may be severe in 5% of affected persons. Neuropathic pain represents the eighth most frequent diagnosisin neurology units, with a prevalence of 3.88% (95% CI: 3.54%- 4.22%). The prevalence of neuropathic pain was 2.92% in primary care centres and 6.09% in hospital units. The daily incidence of new neuropathic pain cases was 1.24% (95% CI: 1.05%-1.53%); 1.14% in primary care neurology centres and 1.45% in hospital units. Neuropathic pain is reported to be common

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based on studies from specialty centres and survey studies. The estimated community prevalence of neuropathic pain based on clinical examination (gold standard) was 9.8%. Only the prevalence rate based on self-reporting of nerve pain was higher (12.4%; Montero *et al*.,2005).

Although neuropathic pain could be acute or chronic in nature, most affected patients suffer from persistent pain comprised of different disease-specific symptoms, each of which having different diagnostic characterisations. Subsequently, it remains difficult to accurately approximate the occurrence and frequency of neuropathic pain (Yawn*et al*., 2009). As such, the burden of neuropathic pain on patients and healthcare systems is potentially massive. Patients with neuropathic pain experience a poor health-related quality of life and consume a high level of healthcare resources and funding. Future prioritisation for neuropathic pain treatment funding by healthcare policymakers requires further data to clarify its epidemiology, the burden on the health of patients, and the demand on healthcare budgets (Ceyhan *et al*., 2005). In order to identify novel therapeutics for neuropathic pain andto specifically design compounds for clinical use in treatment models, it is important to recognise newer efficient drugs.

Commonly used anti-depressant drugs such as duloxetine, venlafaxine, and milnacipran, as well as tricyclic antidepressants such as nortriptyline and desipramine improve neuropathic pain either by the reinforcement of descending inhibitory pathways involving serotoninergic and noradrenergic projection neurons, by inhibiting the re-uptake of 5-HT and noradrenaline and increasing their availability in the spinal cord, or by either direct or indirect involvement of the opioid system (Bridges *et al*.,2001). Since relatively little data exists regarding trazodone efficacy in treating neuropathic pain, the presentstudy aims to evaluate the effect of trazodone on the prevention of neuropathic pain induced by sciatic nerve ligation in rats.

METHODOLOGY

Experimental animals

Male albino rats of theWistar strain (weighing 150-200 g) were obtained for the present study from the animal house stock of the Department of Pharmacology. All animals were housed at ambient temperature (22±1°C), relative humidity 55±5%, and 12-12 h light/dark cycle. Animals were allowed free access to standard chow diet and water given *ad libitum*. The study was approved by the Institutional Animal Ethical Committee**.**

Procurement of drugs

Trazodone (50mg) is marketed for the treatment of major depression by the pharmaceutical company Intas under the trade name Trazonil. All the other chemicals such as sodium dodecyl sulphate, acetic acid, thiobarbituric acid, butanol, pyridine, acetone, eosin, and hematoxyline dye were obtained from the local store and were of analytical grade.

Induction of neuropathic pain by partial sciatic nerveligation method

Neuropathic pain was induced in rats by partial sciatic nerve ligation (PSL) method usingSeltzer's model. Rats were first anaesthetisedwith sodium pentobarbitone (60mg/kg i.p.). When the animal lost consciousness, the left sciatic nerve was exposed at mid-thigh level through a small incision and 1/3 to 1/2 of the nerve thickness wastightly ligated with a 7.0 silk suture. The wound was also closed with a single muscle suture and skin clips and then dusted with Aueromycin antibiotic powder (Angela *et al*.,2016; Seltzer*et al*.,1990)

Experimental protocol

Group I (normal control): Rats (n=6) were not subjected to any surgical procedure and were kept for 3 weeks. Behavioural tests were performed on different days, i.e., day 7, 14and 21. Thereafter, all animals were sacrificed and subjected to biochemical analysis for the estimation of malanodialdehyde (MDA) levels and total calcium in sciatic nerve tissue.

Group II (PSL): Rats (n=6) were subjected to a surgical procedure to expose and create four loose ligations to the sciatic nerve. The behavioural tests and

the biochemical parameters were assessed as per the method described for Group I.

Group III (trazodone 5mg/kg + PSL): Trazodone (5mg/kg) was administered for 21 days(starting from day 1) in rats (n=6) subjected to PSL. The behavioural tests and biochemical parameters were assessed as per the method described for Group I.

Group IV (trazodone 10mg/kg + PSL): trazodone (10mg/kg) was administered for 21 days (starting from day 1) in rats (n=6) subjected to PSL. The behavioural tests and biochemical parameters were assessed as per the method described for Group I.

Group V (Fluoxetine 10mg/kg + PSL): Fluoxetine (10 mg/kg) was administered for 21 days (Starting from day 1) in rats (n=6) subjected to PSL. The behavioural tests and biochemical parameters were assessed as per the method described for Group I.

Behaviouralstudies

Hot plate test

Thermal hyperalgesia (Eddy *et al*.,1953) was assessed by placing individual animals on a hot plate (Eddy's Hot Plate maintained at 55 ^{O}C atweekly intervals on day 7, 14 and 21) following PSL.The latency to first sign of paw licking or jumping response to avoid thermal pain was taken as an index of pain threshold. A cut-off time of 15 s was maintained throughout the experimental protocol.

Cold allodynia

Coldallodynia of the hind paw was assessed using the acetone drop method as described by Vogel *et al*. (1997) with slight modifications to assess reactivity to non-noxious cold chemical stimuli. The rats were placed on the top of a wire mesh grid, allowing access to the hind paws. Acetone (0.1 ml) was sprayed on the plantar surface of the left hind paw of each rat. Cold chemical sensitive reactionsof either paw licking, shaking or rubbing the left hind paw were observed and recorded as paw lifting duration over a 20 s test period(Vogelaar *et al*., 2004).

Mechanical hyperalgesia

Mechanical hyperalgesia was assessed by the pinprick test (Deuis *et al*.,2017). The surface of the injured hind paw was touched with the point of abent gauge needle (at 90° to the syringe) at an intensity sufficient to produce a reflex withdrawal response in normal non-operated animalsbut insufficient to penetrate the skin. The duration of paw withdrawal was recorded in seconds with a stopwatch. A cut-off time of 20 s was maintained.

Biochemical estimation

Estimation of malanodialdehyde

MDA levels were estimated using 300 µl of 10% trichloroacetic acid added to 150 µl of each sample and centrifuged at 1000 rpm for 10min at 4 °C (Yeon *et al*., 2005;Meena*et al*.,2011). A total of 300 µlof the supernatant were transferred to a test tube and incubated with 300 µl of 0.67% thiobarbituric acid at 100 °C for 25 min. The mixture was allowed to cool on water for 5 min. The resulting pink-stained TBARS were determined usinga spectrophotometer at 535 nm.

Estimation of total calcium

Total calcium levels were estimated in the sciatic nerve using sciatic nerve homogenate mixed with 1 ml of trichloroacetic acid (4%) in ice-cold conditions and centrifuged at 2000 rpm for 10 minutes. The clear supernatant was used to estimate total calcium ion by atomic emission spectroscopy at 556 nm.

Statistical analysis:

All values were expressed as mean ±S.E.M. All data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's T-test.

RESULTS

Effect of trazodone on thermal hyperalgesia in partial sciatic nerve ligated rats

In the present study, PSL resulted in a significant ($p< 0.05$) development of thermal hyperalgesia (jumping or licking time latency) noted by a decrease in left hind paw withdrawal threshold compared to the sham group as shown in the figure 1. Administration of trazodone (5 and 10 mg/kg, i.p.) attenuated a PSL-induced decrease in the nociceptive threshold for thermal hyperalgesia, which was less effective compared to the standard.

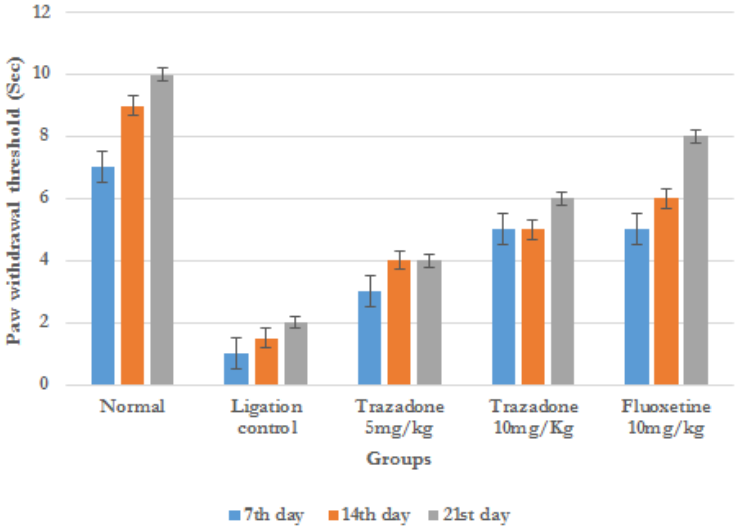

FIGURE 1 - Effect of trazodone on thermal hyperalgesia in partial sciatic nerve ligated rats.

Effect of trazodone on mechanical hyperalgesia in partial sciatic nerve ligated rats

PSL was associated with the development of mechanical hyperalgesia, as reflected by an increase in hind paw withdrawal durationwhen compared to the sham group. (Figure 2) Treatment with trazodone (5 and 10 mg/kg i.p.) attenuated the PSL-induced increase in withdrawal duration of the hind paw in response to noxious mechanical stimuli less effectively when compared to thestandard drug.

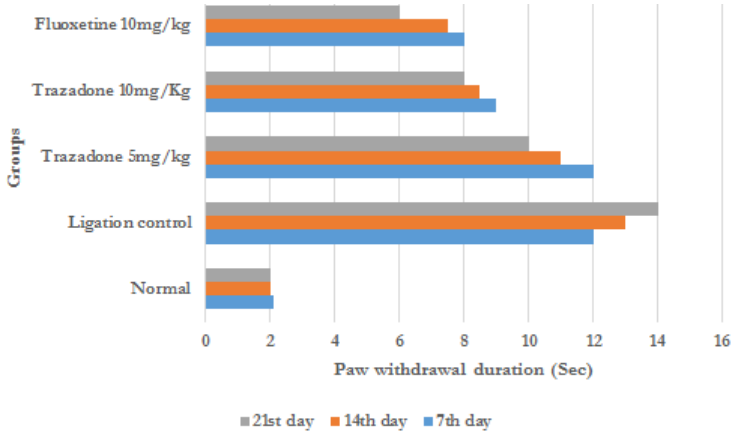

FIGURE 2 - Effect of trazodone on mechanical hyperalgesia in partial sciatic nerve ligated rats.

Effect of trazodone on cold allodynia in partial sciatic nerve ligated rats

PSL resulted in a significant ($p< 0.05$) development of cold allodynia, as reflected by an increase in the duration of hind paw withdrawal when compared to the sham group. Treatment with trazodone (5 and 10mg/kg i.p.) significantly attenuated the PSL-induced increase in the withdrawal duration of the hind paw in response to non-noxious cold stimuli as shown in the figure 3.

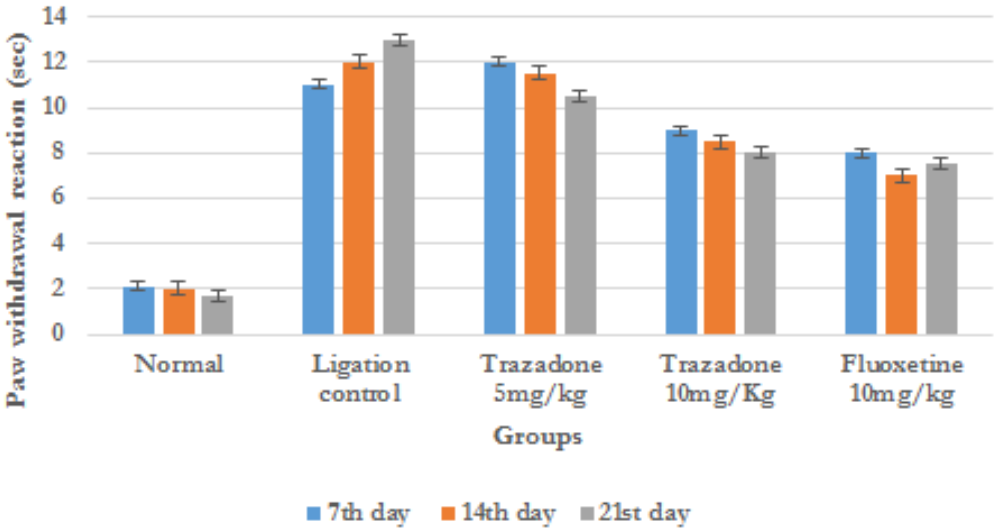

FIGURE 3 - Effect of trazodone on cold allodynia in partial sciatic nerve ligated rats.

Effect of trazodone on histopathological changes

The PSL method exhibited significant histopathological changes in the transverse section of neuropathic pain induced groups, including nerve derangement, axonal swelling and an increase in the number of Schwann and satellite cells. Administration of trazodone (5mg/kg and 10mg/kg i.p.) significantly attenuated PSL-induced fibre derangement, swelling of nerve fibre and activation of neuroglial cells (satellite cells and Schwann cells) as markers of histopathological alteration.

Figures 4A and 4E show normal fibre arrangements. In Figures 4B and 4D, the black arrows show fibre derangement, swelling of nerve fibre and the presence of activated satellite cells and Schwann cells. In Figures 4C and 4E, attenuation of PSL-induced swelling of nerve fibres by trazodone (5 and 10 mg/kg) and standard treatment groupsare observed, respectively.

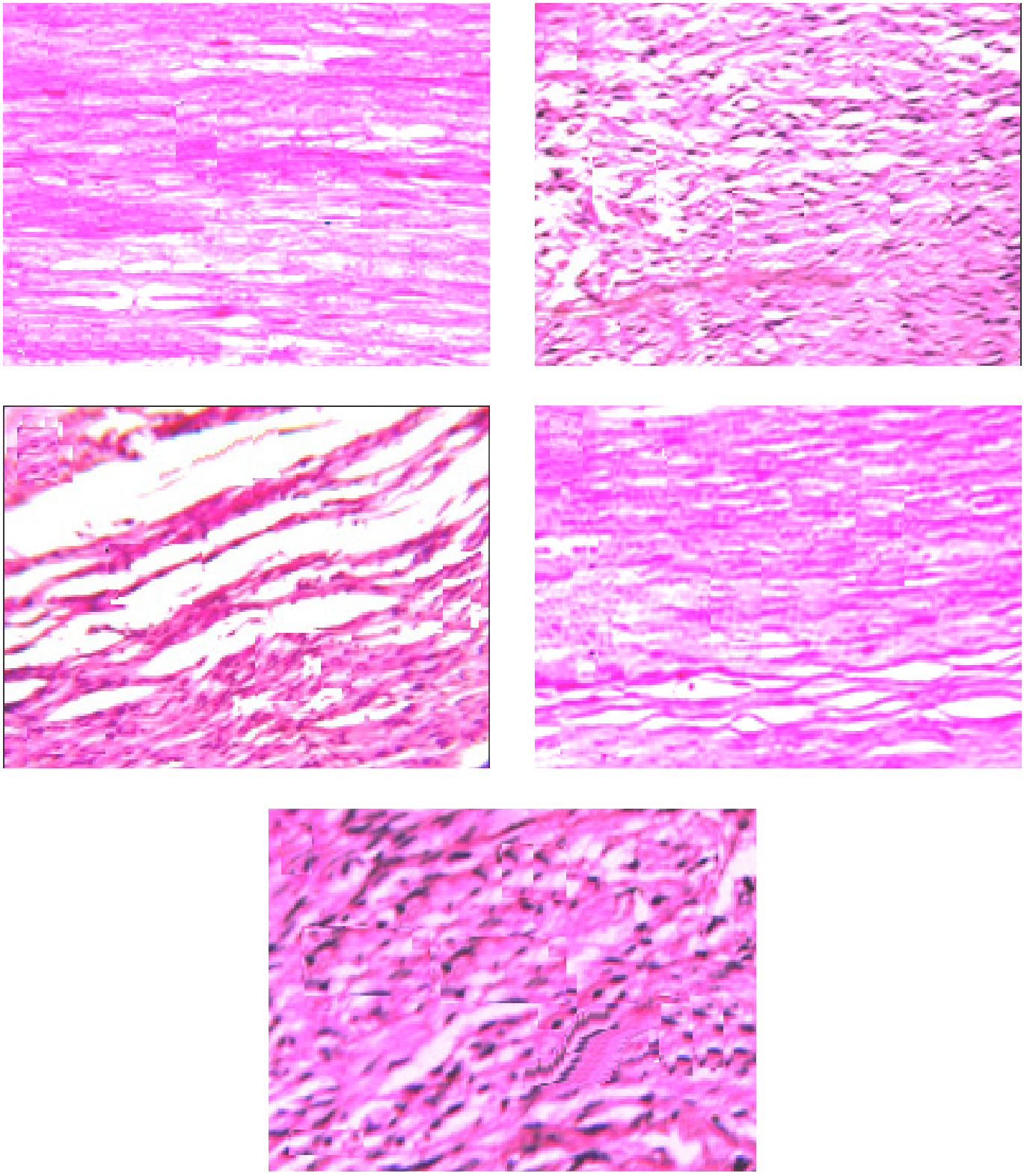

FIGURE 4(A–E)- Effect of trazodone on partial sciatic nerve ligation-induced histopathological changes.

Effect of trazodone on biochemical parameters

PSLincreased the oxidative stress markers and total calcium content as reflected whencompared to the sham group. Treatment with trazodone (5mg and 10 mg/kg i.p.) significantly diminished the PSL-induced increasein oxidative stress markers (Figure 5A) and total calcium levels (Figure 5B).

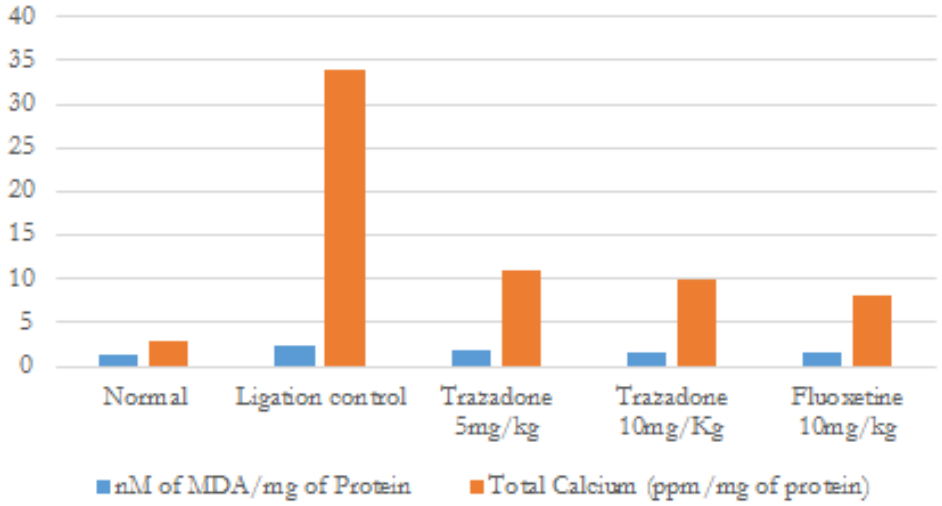

FIGURE 5 - Effect of trazodone on oxidative stress markers and total calcium.

DISCUSSION

Neuropathic pain is a debilitating disease afflicting a wide population. Peripheral nerve injury produces a persistent neuropathic pain state characterised by spontaneous pain, allodynia and hyperalgesia (Obermann, 2019). In the present study, we assessedunilateral sciatic nerve ligation-induced behavioural and biochemical alterations in rats, and it was determined that unilateral ligation of the sciatic nerve in rats produced ipsilateral cold allodynia, thermal hyperalgesia, mechanical hyperalgesia and oxidative damage in the sciatic nerve (Gilron *et al*.,2006; Qin*et al*.,2019;Wallace *et al*.,2008).

In the present study, trazodone(5mg/kg and 10mg/kg) attenuated sciatic nerve ligation (i.e., PSL-induced behavioural [i.e., thermal and mechanical sensation], biochemical [i.e.,lipid peroxidation and total calcium] and histopathological [i.e.,axonal degeneration] changes). The behavioural alterations started on day 7 following the partial ligation of the sciatic nerve, followed by days 14 and 21.

During the present study, trazodonetreatment significantly reversed thermal hyperalgesia, mechanical hyperalgesia and cold allodynia in sciatic nerve-ligated animals, thereby suggesting its therapeutic potential in the effective treatment and management of neuropathic pain. These findings suggest that trazodoneplays an important role in pain regulation at the central and peripheral level. Moreover, it was observed that alow dose of trazodonehas a less significant effect than a high dose.

In response to nerve injury, the initial steps of inflammatory reactions involve the release of pro-inflammatory mediators from the resident macrophages, Schwann cells and area adjacent to the nerve lesion. It has been documented that the sustained activation of peripheral nociceptors leads to the hypersensitivity of the primary afferent neurons and central sensitisation of the dorsal horn neurons (Brink*et al*., *2006*).

Furthermore, PSL was associated with elevated oxidative stress, MDAlevels and total calcium contentin the present study. It has also been documented that oxidative stress and increased calcium levels play a critical role in neuropathic pain. However, treatment with trazodoneattenuated the PSL-associated increase in oxidative stress and calcium levels. Trazodoneadministration exhibited an antioxidant effect

and also decreased calcium levels. Notably, free radicals have been well documented to increase calcium levels. Therefore, the observed decrease in calcium levels with trazodonemay possibly be attributed to its antioxidant effects (Brink *et al*., *2006*;Gurpreet *et al*.,2010;Perez *et al*.,2004).

In the present study, PSL resulted in significant histopathological changes assessed in transverse sections of the sciatic nerve. In transverse sections, nerve derangement, axonal swelling and an increased number of Schwann and satellite cells were also noted. Administration of trazodone(5mg/kg and 10mg/kg) significantly attenuated PSL-induced fibre derangement, the swelling of nerve fibre and activation of neuroglial cells (satellite cells and Schwann cells) as markers of histopathological alterations.

CONCLUSION

The results of the present study suggest that the administration of anti-depressantsresults in anti-nociceptive activity in a PSL model of neuropathic pain. The current study exhibited the efficacy of trazodone in the prevention of neuropathic pain induced by PSL in rats. However, this study has some limitations. First, the sample sizes are relatively small. Second, there is variability in the selection of animals, and it is evident that there are variations in neuropathic pain that could potentially impact the results. In conclusion, trazodone may be used as an alternative to analgesics for the symptomatic treatment of neuropathic pain. Although the present evidence highlights the efficacy of trazodone, further research is required to expand on these findings. The present findings suggest that trazodone may be effective for preventing neuropathic pain,though future research can further elucidate the treatment of nociception in clinical practice.

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