International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 583-593
Research Article |Pharmaceutical Sciences| Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

EFFECT OF LACOSAMIDE IN STREPTOZOTOCIN-INDUCED DAIBETIC NEUROPATHIC PAIN

Giram Padmaja S.*, Upasani Chandrashekhar D., and Bhusnure O.G

School of Pharmacy, Department of Pharmacology, SRTMU, Nanded (MS), India.

***Corresponding Author Email: varshagiram@gmail.com**

ABSTRACT

Objective: *Objective of the present study was to investigate anti-nociceptive effect of lacosamide in STZ-induced diabetic rats.* ***Material & Methods:*** *Antinociceptive effect of lacosamide (5, 15 & 45 mg/kg body weight i.p.) was evaluated in the STZ-induced diabetes rat model (Streptozotocin 55 mg/kg i.p.) in total different five groups. Eddy's hot plate and tail immersion test were performed on 1st, 2nd, 3rd and 4th weeks (On day 0, day 7, day 14, day 21 and day 28) of experiment to assess thermal hyperalgesia and cold allodynia respectively. Thermal allodynia evaluated by hot plate (at 45^{0}C±0.50C); Thermal hyperalgesia evaluated (at 55^{0}C±0.5^{0}C) and tail emersion (Cold water 10^{0}C±0.5^{0}C and hot water 55^{0}C±0.5^{0}C) method; and rota rod test was conducted to examine motor function and also tail flick and pin prick methods as measures of neuropathic pain. Further the dose-dependent improvement was observed in thermal hyperalgesia cold allodynia.* ***Results and Conclusion:*** *A significant degree of thermal (Allodynia and hyperalgesia) and mechanical hyperalgesia (p ≤0.05) was produced in all the treatment animal groups. There was also decrease in the grip strength in diabetic rat which indicates induction of neuropathy or nerve damage. The result of the present study indicates that the lacosamide demonstrate that significant (p<0.05) antiallodynic and antihyperalgesia effects on STZ-induced diabetic rats. Lacosamide increase the grip strength, licking time, withdrawal latency and loss of pain perception, prevention of nerve damage in treatment demonstrates its protective effect in diabetic neuropathy.* ***Conclusion:*** *Lacosamide was effective in reducing both the thermal and mechanical hyperalgesia means it has shown good efficacy in different models of STZ induced neuropathic pain.*

KEY WORDS

STZ- Diabetic Neuropathy, Lacosamide, Behavioural Methods, Antinociceptive

INTRODUCTION

Pain is defined as an unpleasant sensation and emotional experience associated with actual or potential tissue injury. Everyone at some point has experienced a painful sensation. Pain can cause unwanted physical, emotional and social anguish throughout one's daily life

Painful diabetic neuropathy (PDN) is one of the leading causes of neuropathic pain in humans.[1-3] PDN is a chronic, usually symmetrical sensorimotor polyneuropathy that produces significant morbidity with negative influence on a patient's general activity. Mood, mobility, work, social relations, sleep and overall quality of life.[1-3] Treatment of PDN is challenging because the mechanism involved are unclear.[4] And the mechanisms of the action for drugs used to treat neuropathic pain have not been fully elucidated. Making it difficult to match the type of pain to the most appropriate medication. Pharmacological agents used in the management of PDN include tricyclic antidepressants, selective serotonin and norepinephrin reuptake inhibitors, opoids, and antiepileptic drugs.

Generally, the available treatment options not given total relief, are not effective to all patients, and only about one-third patients may achieve more than 50% pain relief.[5] Anticonvulsants are increasingly important in the management of neuropathic pain with antiepileptic drugs such as lamotrigine, gabapentin and pregabalin demonstrating an analgesic effect in diabetic neuropathy.[1,6-7]

Neuropathic pain is common in the palliative care population; unless adequately treated, the pain can lead to chronic anxiety, depression, and social impairment. Many treatments have been proposed for neuropathic pain; however, it remains underdiagnosed, under-treated, and often requires long-term therapy with risk of adverse effects. Most existing drugs provide only temporary relief from pain and must be taken consistently on a daily or weekly basis. Current findings provide a greater understanding in the mechanism of chronic pain, which can ultimately contribute to development of new target specific medications for alleviating this debilitating disease state.

Lacosamide the R-enantiomer of l-acetamido-N-benzyl-3-methoxypropionamide. Was synthesized as an anticonvulsive drug candidate. Animal model studies have shown that Lacosamide has antiepileptic and antinociceptive efficacy. Including efficacy in the sterptozotocin-induced rat model of diabetic neuropathic pain. Where it showed equivalent or greater efficacy on measures of allodynia and hyperalgesia to that of other antidepressant or anticonvulsant drugs.[8-9] It is absorbed rapidly and completely after oral administration (bioavailiabilty-100%) with minimal protein binding.[10-11]

Though gabapentin is very effective in diabetic neuropathic pain, it is lamotrigine which is superior to gabapentin in cancer chemotherapy induced neuropathic pain. This may be probably due to difference in the analgesic action of different antiepileptics. Also, the underlying mechanisms of neuropathic pain of different etiologies need to be understood. While it is always exciting to see a compound with a novel mechanism of action show great efficacy in a rodent model of inflammatory or neuropathic pain, the obvious ultimate goal is to identify novel compounds that are found to be safe and effective in humans. A major challenge faced in the development of new analgesics is establishing confidence in the predictive validity of preclinical models of pain.

With the development of animal models and improved understanding of pathophysiology of neuropathic pain, the efficacy of different groups of drugs is being assessed. Tricyclic antidepressants (TCAs), often the first choice has significant side effects[12] and antiepileptics are partially effective.[13]So it is difficult to select a right drug in different types of neuropathic pain.

Multitude of neuropathic pain states & the complex pathophysiological mechanisms involved means that a drug effective in one pain state may not be effective in another; and the response produced may also not be adequate. But no drug, whether conventional or non-conventional, is fully effective in the treatment of this condition, and a drug that shows good efficacy in one neuropathic pain state may be ineffective in another. Thus, there is a continuing need to evaluate newer drugs with different mechanisms of action in various models of neuropathic pain. Among the behavioural tests used thermal hypo or hyperalgesia is commonly used to assess the sensory perception disturbances in neuropathic pain.

With the ever-increasing demand for newer treatment modalities, Lacosamide have recently been investigated for its role in treatment of painful diabetic neuropathy in a rat but is in unclear and observer bias. With this background, a study has been undertaken to compare the efficacy of antiepileptic drug (lacosamide) for effectiveness in neuropathic pain model by STZ induced diabetes rats.

2 MATERIAL AND METHODS

2.1 Animals

Experimental animals include wistar rats (180-250g either sex) were used for the evaluation of the neuropathic pain activity. The animals were obtained from Wockhardt Research Centre, D-4 MIDC, Chikalthana, Aurangabad. All animals were housed for at least two weeks in the laboratory animal room prior to study. The selected animals were housed in polypropylene cages in the standard environmental conditions (20-25°C), 12:12 light: dark cycle, fed with standard rodent diet (VRK Nutritional Solution, Sangali) and water *ad libitum*.

The experiments on animals were conducted in accordance with the international accepted principle for laboratory animal use and the experimental protocols duly approved (Proposal Number: CPCSEA/CBPCL/ IAEC/2015-16/03.) by the institutional animal ethical

committee (IAEC) of Channabasweshwar Pharmacy College, Latur (MS) India

2.2 Drugs and Reagents

Lacosamide (5, 15 and 45 mg/kg body wt.) procured as a research gift sample from Micro Lab Pvt Lts, Mumbai, Streptozotocine (STZ) injection 98% from SISCO Research laboratories Pvt. Ltd. All the chemicals were purchased from S. D. Fine Chem Pvt. Ltd, Mumbai, India.

2.3 Experimental design and development of diabetes in the rat

The rats were randomly divided into two groups, the STZ-induced group (n=24) and the control group (n=06). After one week of acclimatization, the STZ-induced rat model was established by intravenous (i.p.) injection of buffered solution of streptozotocin (55 mg/kg body weight) in freshly prepared 0.1 mol/l citrate buffer (pH 4.5). Control animals received an equivalent volume of citrate buffer solution. Seventy-two hours later, the plasma glucose levels were determined by the glucose oxidase method using the one touch ultra-blood glucose meter (Mfg. by Flextronic Industrial Co. Ltd. Shenzhen, Guangdong, marketed by LifeScan, Johnson & Johnson Pvt. Ltd, Mumbai) and rats with the blood glucose more than 16.7 mmol/L were considered to be successfully induced for diabetes. Animals with blood glucose levels <14.3 mmol/l (260 mg/dl) were immediately excluded from the study. In this study care was taken during the induction of diabetes in order to avoid illness that was too severe. The body weight and general health was monitored, and all animals demonstrated appropriate behavior during the entire study. After the successful construction of the rat diabetic models, the rats in the STZ-induced group were divided into four treatment group, one disease control as well as normal control.

Six groups, each group comprising of six Wistar rats, were used in the present study.

- **Group I (Normal control group):** Rats were administered with normal saline (1ml/kg 0.9% w/v of sodium chloride; i.p. once a day) for 28 days.
- **Group II (STZ-control group):** Rats were administered with Streptozotocin (55 mg/kg; i.p. once only to induces diabetes)
- **Group III (LCM 5):** Rats were administered LCM (5mg/kg; i.p.) 30min before start behavioral study.
- **Group IV (LCM 15):** Rats were administered LCM (15mg/kg; i.p.) 30min before start behavioral study.
- **Group IV (LCM 45):** Rats were administered LCM (45mg/kg; i.p.) 30min before start behavioral study.

2.4: Behavioral assessment

2.4.1. Hot plate test (Thermal allodynia and Thermal hperalgesia)

Hot Plate analgesia meter was used to determinate the central component of nociception. Animals were individually placed on a Hot Plate Analgesia Meter (Orchid Scientific and innovative India Pvt. Ltd., Nashik) maintained at constant temperature at 45^0C ±0.50C (Thermal allodynia) and at 55°C± 0.5^0C (Thermal hyperalgesia). 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 period of 30 Sec and 15 sec respectively was observed to avoid damage to the paws.[14]

2.4.2 Tail immersion (*Cold and hot allodynia*) test

Diabetic thermal hyperalgesia was assessed using tail immersion test. After adaptation, rat tail was immersed in cold (10^0C±0.5^0C) & hot water (55°C±0.5^0C) and the tail flick response latency (withdrawal response of tail) was observed as the end-point response. Each experiment was repeated 3 times for each animal with an interval of 5 min and its average was reported. Meanwhile, the cut-off time was 20 sec and 15 sec respectively.[15-16]

2.4.3 Acetone test (Thermal allodynia) [17]

Cold chemical thermal sensitivity was assessed using acetone drop method as described by Choi et al., with modification. Rats were placed in a glass mesh cage and allowed to habituate for approximately 20 minutes in order to acclimatise them for the new environment. Freshly dispensed acetone drops (50µL) was applied gently on to the mid plantar surface of the hind paw. Cold chemical sensitive reaction with respect to either paw licking, shaking or rubbing the hind paw and brisk foot withdrawal (typically 2–5sec after application) was recorded as a positive response (nociceptive pain response) were as absence or delay of these responses were considered as anti-nociceptive effect. The responses were measured with a digital stopwatch. For each measurement, the paw was sampled three times for both paws and the mean was calculated. The interval between each application of acetone was approximately 5 minutes.

2.4.4 Motor co-ordination[18-19]

Rota-rod test was performed to investigate the change in motor coordination. The effect on motor performance was evaluated using an accelerating rota rod in which normal rats were placed on a rotating drum

with the speed increasing from 4 to 20 rpm over 5 min, forcing them to walk forward to avoid falling. The time(s) to falling was measured. Training sessions were carried out 1 and 2 days prior to the experiments, with three trials on each day. On the experimental day, a baseline response was obtained and the effects on motor coordination. Rats were placed on a rotating rod and the latency to falling was measured for up to 5 min according to the method described previously.

2.4.5 Pin prick test (Mechanical hyperalgesia)[20]

Mechanical hyperalgesia was evaluated by the pin prick test as described in the method by Erichsen & Blackburn-Munro. Briefly, the plantar surface of the left hind paw was touched with the point of the pin (at 90 angles) at intensity sufficient to produce a reflex withdrawal response in normal rats, but at an intensity which was insufficient to penetrate the skin in all groups. The duration of the paw withdrawal was recorded in seconds. A cut-off time of 20 s was maintained.

3. Statistical analysis

All statistics tests were conducted using Graph Pad Prism (version 7) software. The values are expressed as mean ± SEM of six animals. Values with $P < 0.05$ or less were considered as statistically significant. Differences between the studied groups were examined for statistical significance as regards the various parameters using the analysis of variance test. This test is used to find a significance difference between more than two groups. Data were tabulated and represented graphically.[21]

RESULT

3.1. Development of body weight and blood glucose levels in streptozotocin-treated rats

The streptozotocin-treated animals had 25% less body weight than the control rats on day 28th and 24% less on day 20 (Fig.1a). The average blood glucose levels of the streptozotocin-treated animals were 350 mg/dl on day 7 and 420 mg/dl on day 28 compared to 116 mg/dl of the control animals (Fig. 1b). The general health was monitored strictly and even though the diabetic rats had a slightly reduced body weights all animals demonstrated normal behavior during the entire study. (Fig.No1 a & b)

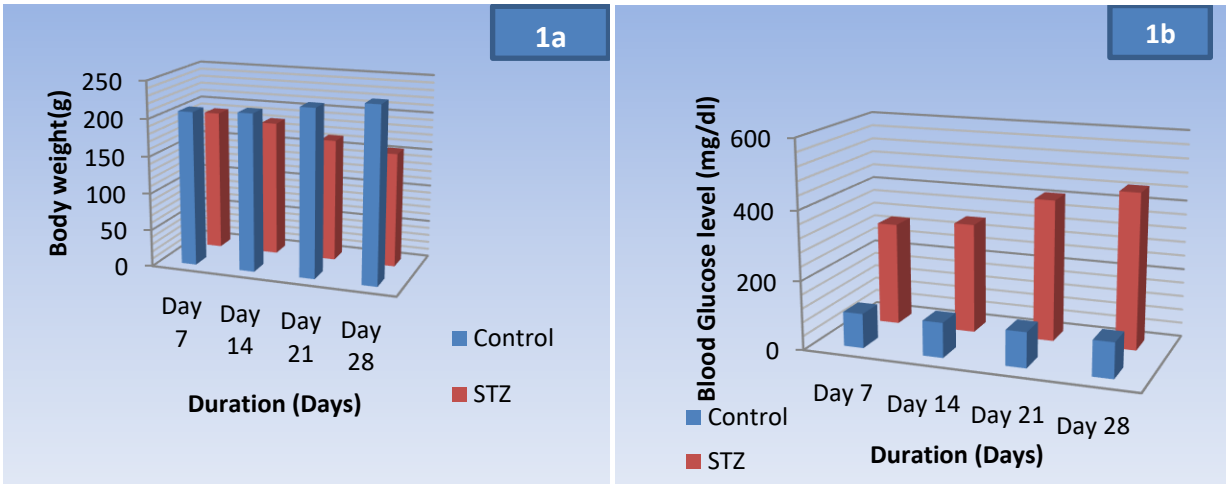

Fig. 1a. Body weight and blood glucose levels in control (n=06) and streptozotocin (STZ)-treated rats (n=24) on day 7, 14, 21and 24 after STZ-treatment shown in diagram 1a & 1b.

3.2 Hot plate test (Thermal allodynia and Thermal hyperalgesia)

***3.2.1* Thermal allodynia (45^{0}C±0.5)**

After four weeks of diabetes induction, the nociceptive threshold was significantly lower ($p<0.01$) in diabetic rats in all groups as compared with normal control. Thermal Hyperalgesia was evident in streptozotocin treated animals since paw withdrawal latency was significantly shorter ($p<0.01$) than that of normal animals after four weeks of diabetes induction. The groups treated with lacosamide (5 mg/kg, 15mg/kg and 45 mg/kg, i.p) showed significant increase in reaction time when compared with diabetic control. The therapeutic combination groups of lacosamide (45 mg/kg, i.p) showed much more significant ($p<0.01$)

increase in reaction time when compared with diabetic control (Fig 2).

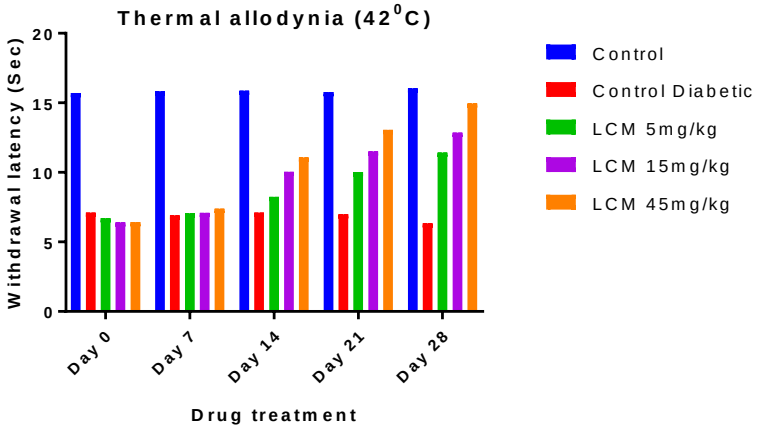

Figure 2: Effect of lacosamide on thermal allodynia test (at 42^{0}C) (Tail withdrawal threshold). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ group; p > 0.05 non-significant versus normal control group & STZ group.

3.2.2 Thermal hperalgesia (at 55^{0}C±0.5)

The groups treated with lacosamide (5 mg/kg, 15mg/kg and 45 mg/kg, i.p) showed significant increase in reaction time when compared with diabetic control. The therapeutic dose of lacosamide (45 mg/kg, i.p) showed much more significant (p<0.01) increase in reaction time when compared with diabetic control on 28th day (Fig 3).

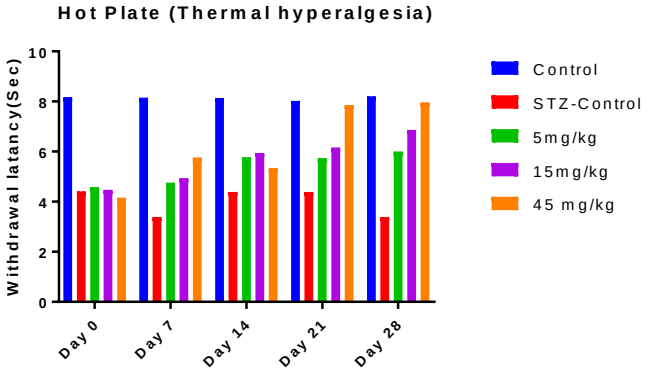

Figure 3: Effect of lacosamide on thermal hyperalgesia test (at 55^{0}C±0.5) (Tail withdrawal threshold). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ group; p > 0.05 non-significant versus normal control group & STZ group.

3.3 Tail immersion (Cold and hot allodynia) test

3.3.1 Hot water test (at 55^{0}C±0.5)

The diabetic control group showed significant decrease (p<0.01) in reaction time when compared with normal control. The groups treated with lacosamide Lacosamide (15 and 45 mg/kg; i.p.) showed significant increase in reaction time (Tail withdrawal latency) when compared with diabetic control. The therapeutic effect of lacosamide on 28th day (45mg/kg,i.p) showed more significant (p<0.01) increase in reaction time (Tail withdrawal latency) when compared with diabetic control(Fig. 4).

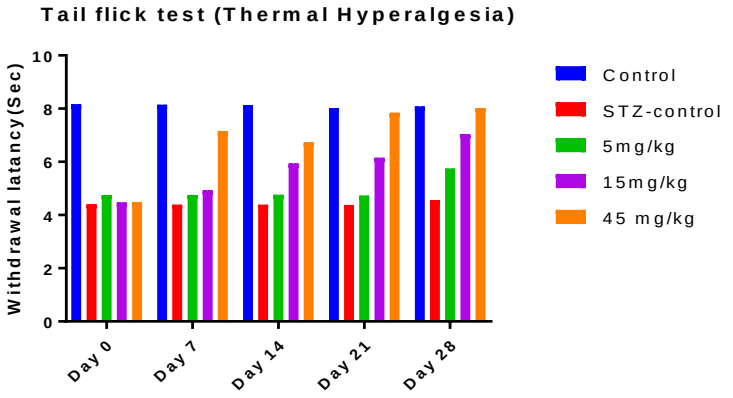

Figure 4: Effect of lacosamide on Tail immersion hot water test (at 55^{0}C±0.5) (Tail withdrawal threshold). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ group; p > 0.05 non-significant versus normal control group & STZ group.

3.3.2 Cold water test (10 ^{0}C ± 0.5)

Pain elicited by cold is the major feature of many neuropathic pain states. Especially in cold allodynia normal cool stimuli elicits pain in animal models. For testing cold allodynia two standard animal models, namely Acetone drop test and Tail immersion test were used in the present study.

Streptozotocin-treated animals which received the vehicle exhibited a very short mean threshold latency in the cold bath test (approximately 6 s) in contrast to control/vehicle-treated animals (>15 s) which indicates that cold allodynia developed (Fig. 3). Treatment of streptozotocin-animals with lacosamide 15 and 45 mg/kg (i.p.) produced statistically significant increases in the threshold latency (P<0.05, Dunnett's test). In fact, lacosamide at 45 mg/kg doses produced full reversal of streptozotocin-induced cold allodynia. However, although the threshold latency was greater at the 5, 15 and 45 mg/kg dose of lacosamide than that in vehicle-treated streptozotocin-animals, the difference was not statistically significant. (Figure 6).

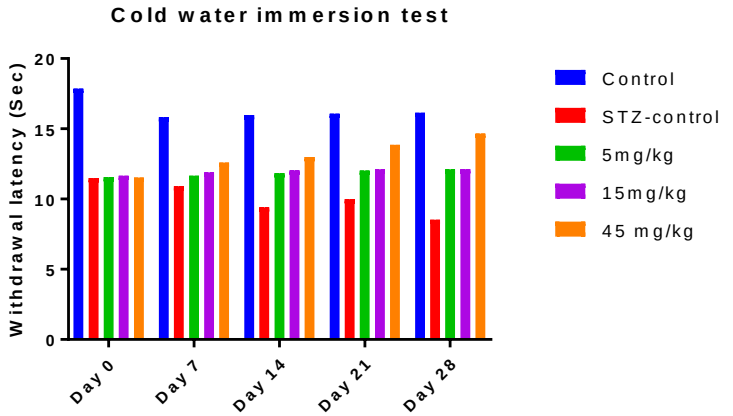

Fig.5.: Effect of lacosamide (i.p.) on paw withdrawal latency in cold water model (10^{0}C±0.5). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ control group; p > 0.05 non-significant versus normal control group.

3.3 Acetone test (Thermal allodynia)

In this method the brisk foot withdrawal response, paw licking, shaking or rubbing the hind paw and its frequency was considered as positive response. lacosamide treated group resulted a significant reduction in foot withdrawal response when compared

to STZ-control group (p< 0.001). Furthermore, in lacosamide group, the foot withdrawal response re-appeared at dose of 45mg/kg on 28th day of treatment. Lacosamide treated group resulted inhibition of foot withdrawal response, paw licking, shaking or rubbing the hind paw noticed in all periods of observation by dose dependence manner(Fig.6).

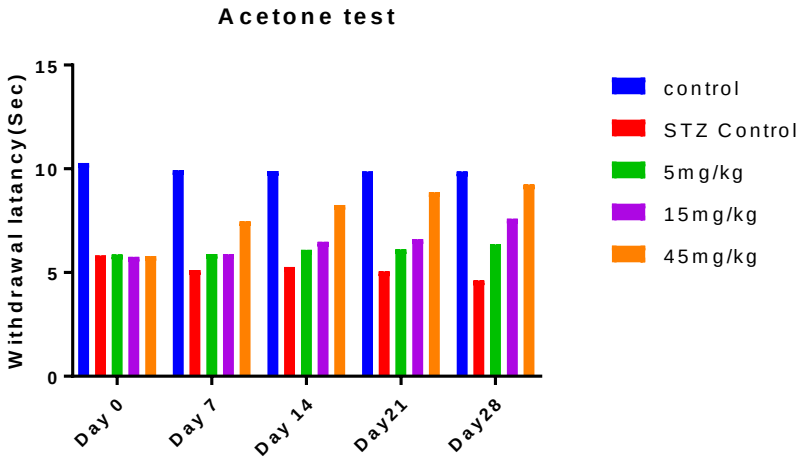

Fig.6.: Effect of lacosamide (i.p.) on paw withdrawal latency in cold water model (10^0C)**. Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ control group; p > 0.05 non-significant versus normal control group.**

3.4 Rota rod Model

After four weeks of diabetes induction muscle spindle get damage and can lead to deficits such as motor incordination. In rota rod test fall down latency time of animals on rotating rod was calculated. The diabetic control group animals showed significant decrease in fall latency time (p<0.01) when compared with normal group. The groups treated with lacosamide 5, 15 and 45 mg/kg,i.p showed significant increase in fall latency time (p<0.01) when compared with diabetic control. Therapeutic effect of standard gabapentin at dose of 30mg/kg significantly (P<0.01) attenuated STZ-induced decrease in fall off time. However, LCM 45 mg/kg i.p. did not produce any significant change in fall off time as compared to the normal control group (Figure 7).

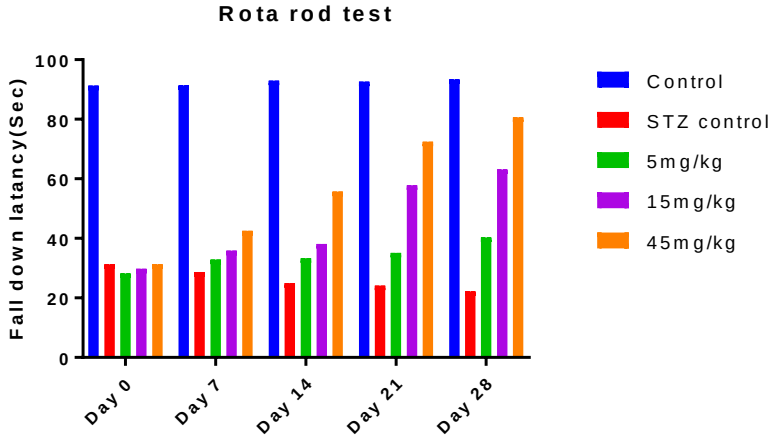

Figure 7: Effect of lacosamide on muscle grip strength (i.e. Fall off time). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ control group; p > 0.05 non-significant versus normal control group.

3.5 Pin prick test (Mechanical hyperalgesia)

In this method Mechanical hyperalgesia was evaluated by the pin prick test and withdrawal latency was recorded as second. Treatment of lacosamide at dose 45mg/kg has shown highly significant activity as compare to 5 and 15 mg/kg. All the treatment dose are shows the positive response and values are scientifically significant ($p>0.05$) compare to the STZ control group (Fig 8).

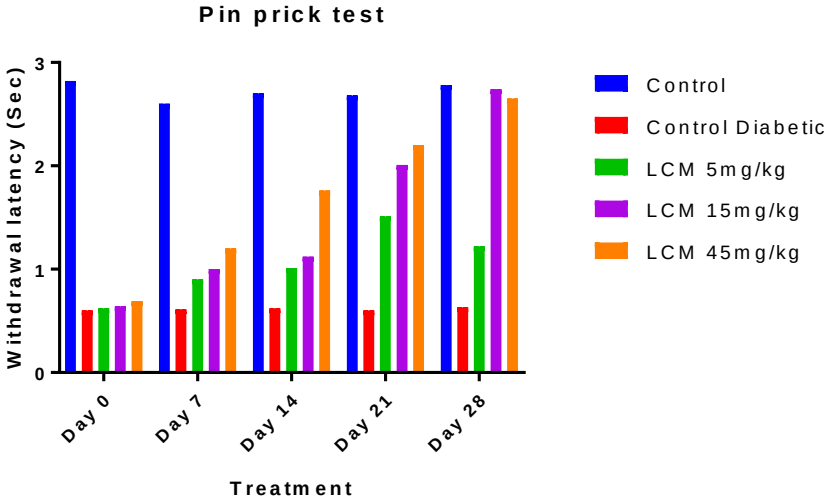

Figure 8: Effect of lacosamide on pinprick test (i.e. Paw withdrawal threshold). Data are expressed as mean ± SEM, n = 6 rats per group. p < 0.01 versus normal control group; p < 0.01 versus STZ control group; p > 0.05 non-significant versus normal control group.

DISCUSSION

Chronic pain affects the life of millions of people. Neuropathic pain is a debilitating condition arising from injury to the somatosensory neurons which triggers the development of allodynia and hyperalgesia. Several conditions such as traumatic accidents, surgery and diseases affecting peripheral and central nervous systems contribute to etiopathogenesis of the neuropathic pain. Experimental studies reveal inflammation is the main cause of neuropathic pain. The inflammatory cytokines and oxidative stress along with allogeneic mediators released subsequent to a nerve injury induce neuropathic pain through sensitization of the nociceptive receptors.

There are very limited treatments available for neuropathic pain, and a lot of patients use opioids, In the long term, these can lead to addiction and severe side effects, including dependence and tolerance, opioid-induced hyperalgesia (the pain becomes even worse), and risk of death. For these reasons, identifying novel analgesics is of keen interest in the medical field today. Neuropathic pain is an important public health problem for which only a few treatments are available. Current treatments have deleterious side effects. There is a growing need for studies to evaluate the most potent drugs or combinations for the management of DN to maximize pain relief and improve quality of life. The novel strategy for targeting protein interactions that regulate the N-type voltage-gated calcium (CaV2.2) channel as an alternative to direct channel block: peptides uncoupling CaV2.2 interactions with the axonal collapsin response mediator protein 2 (CRMP2) were antinociceptive without effects on memory, depression, and reward/addiction. The development of novel CaV2.2-targeted drugs with improved efficacy and therapeutic index is highly desirable.[22]

Animal models performed by different laboratories reflect differences in their characteristics, like differences in the induction of hyperalgesia. [23-24] In vitro experiments demonstrate that the mechanism of action of lacosamide appears to be novel. Lacosamide does not bind to a range of receptors, ion channels, transporters or enzymes. Lacosamide may act in a manner unlike currently available anticonvulsants and antidepressants. A novel mode of action may be responsible for the results of this study which suggest that lacosamide may be more potent and/or more efficacious in animal models of polyneuropathy than the other anticonvulsants studied lamotrigine (sodium

channel inhibitor, levetiracetam (neuronal synchronization modulator, antidepressants like amitriptyline (tricyclic reuptake inhibitor of noradrenaline and serotonin, pregabalin (calcium channel modulator and venlafaxine (serotonin- and weak noradrenalin reuptake inhibitor.[25-27]

Diabetic neuropathy is characterized by clinical features like allodynia and hyperalgesia due to elevated nociceptive response. Similar symptoms are exhibited by STZ-induced diabetic animals STZ is an antibiotic extracted from *Streptomyces acromogenes* and is diabetogenic due to a selective cytotoxic action upon pancreatic β cell. STZ injected rats exhibit clinicopathological features including biochemical, oxidative, and metabolic changes which also presented in humans [28-30]

It has been reported earlier that STZ-induced diabetic neuropathic pain is characterized by hyperalgesia and allodynia[31-32] and was also found in the present study following STZ injection. A decrease in pain threshold was observed in STZ-diabetic rats using a mildly noxious stimulus such as mechanical force. Moreover, there was also a similar increase in thermal hyperalgesic activity in diabetic rats as compared with normal rats when subjected to thermal stimuli. In the present investigation, we found that administration of LCM dose dependently reversed STZ-induced thermal hyperalgesia and mechanical allodynia. it has identified a novel dual mode of action for lacosamide: the selective enhancement of sodium channel slow inactivation and the modulation of CRMP-2 (collapsin-response mediator protein 2), these novel modes of action could be the basis for the efficacy of lacosamide in the treatment of epilepsy and diabetic neuropathic pain and potentially slow or even stop the progression of the diseases. “Based on the proposed modes of action, patients might could get a significant benefit in the treatment of their diseases: Lacosamide seems not only to be an efficacious treatment option in epilepsy and neuropathic pain but might have the potential to directly affect the progression of the diseases”

In the present study decrease in the pain threshold was observed with mildly noxious stimulus after streptozotocin induced diabetic neuropathy but this threshold was improved by using Lacosamide individually.

The percentage increase in reaction time in case of Thermal allodynia and Thermal hperalgesia (Eddy‟s hot plate) shown that more than 75 % increase in case of Lacosamide at dose 45 mg/kg,i.p which was more as compared to Lacosamide at dose 5 and 15 mg/kg,i.p. on day 28.

In tail immersion (Hot water) model lacosamide 45mg/kg,i.p had shown more than 75% increase in tail withdrawal latency which was more as compared to Lacosamide at dose 5 and 15 mg/kg,i.p. on day 28.

In the present study, lacosamide treated group significantly attenuated STZ-induced hyperalgesia as evidenced by increased tail withdrawal latency using radiant heat method for initial two weeks of observation when compared with vehicle and STZ treated group. Furthermore, increase in tail withdrawal latency was also observed in third and fourth weeks of observation as compared to vehicle and STZ treated animals.

In tail immersion (cold water) model lacosamide 45mg/kg,i.p had shown significant increase in tail withdrawal latency which was more as compared to Lacosamide at dose 5 and 15 mg/kg,i.p. on day 14 and day 21. In Tail flick model the % increase in tail withdrawal latency observed that lacosamide 45mg/kg,i.p had shown more than 75% increase in tail withdrawal latency which was more as compared to Lacosamide at dose 5 and 15 mg/kg,i.p. on day 28.

The anti-hyperalgesic activity was evaluated in case of Rota rod model by % increase in fall latency time. lacosamide 45mg/kg,i.p had shown more than 50% increase in fall latency time which was more as compared to Lacosamide at dose 5 and 15 mg/kg,i.p. on day 28.

In the present study, streptozotocin-injected rats had significantly higher blood glucose level, decreased body weight and the nociceptive threshold was significantly lower than non-diabetic rats in hot plate, tail immersion, paw pressure withdrawal tests, indicating that diabetic animals exhibited significant thermal and mechanical hyperalgesia. This condition was reversed following the treatment of Lacosamide at dose 45mg/kg,i.p on 28th day of treatment.

Lacosamide was effective in reducing both the thermal and mechanical hyperalgesia means it has shown good efficacy in different models of neuropathic pain.

The anti-allodynic effect of lacosamide was maximum for a period of 12 days in yet another study, whereas in our study, lacosamide had produced prolonged effect (4 weeks). This was further confirmed by absence of foot

withdrawal, paw licking, shaking or rubbing the hind paw during the observation time of one minute in all the four weeks of the study. The results obtained are consistent with previous findings that lacosamide has anti-nociceptive properties in experimental neuropathy.[34-36] These results suggested that lacosamide has reduced development of diabetic nephropathy in streptozotocin-induced diabetic rats and could that beneficial effect in reducing the progression of diabetic nephropathy. Similar to finding of other anti-epileptic drugs (AEDs) in the treatment of neuropathic pain lacosamide can well established as per previous study and finding. [37-42]

CONCLUSION

These results indicated a loss of pain perception in diabetic rats in all the behavioral models attributed to nerve damage resulting due to the development of diabetes neuropathy. While chronic treatment of lacosamide treatment groups caused an increase in the latency time or withdrawn time dose dependently. Thus, from the result it indicates that chronic treatment of lacosamide prevent progression of diabetic neuropathy in streptozotocin-induced diabetic rats. From this study it was concluded that lacosamide treated animals decreased thermal hyperalgesia and possessed anti-allodynic effect and was most effective in attenuating STZ-induced diabetic neuropathic pain. So, it can be also concluded that lacosamide can be used as ideal drug which could offer a better in treating the diabetic neropathy.

ACKNOWLEDGEMENTS:

Authors wish to thanks to the Dr. Gattani S.G. for providing the facilities necessary to carry out research work. Authors are thankful to Dr. Thonte S.S, Principal, Channabasweshwar Pharmacy College, Latur for constant moral support and encouragement.

REFERENCES

1. Vinick A. clinical review, use of antiepileptic drugs in the treatment of chronic painful diabetic neuropathy. J clin endocrinol metab 2005; 90:4936-45.
2. Boulton AJ, Vinick AL, Arezzo JC, Bril v, Feldman EL, Freeman R, et al, Diabetic neuropathies a statement by the American Diabetes Association, Diabetes care 2005;28:956-62.
3. Schmader KE, Epidemiology and impact on quality of life of post herpetic neuralgia and painful diabetic neuropathy, clin J pain2002;18:350-4.
4. Campbell JN, Meyer RA, mechanisms of neuropathic pain. Neuron 2006;52(1):77-92.
5. Jensen TS, Backonja MM, Hernandez Jimenez s, Tesfaye S, Valnesi P, Ziegler D, New perspectives on the management of diabetic peripheral neuropathic pain. Diab Vasc Dis Res 2006; 3(2):108-19.
6. Attal N, Cruccu G, Haanpaa M, Hansson P, Jwnsen TS. NurmikkoT, et al. EFNS guidelines on pharmacological treatment of neuropathic pain. Eur J Neurol 2006; 13(11):1153-69.
7. Collins SD, Chessell IP, emerging therapies for neuropathic pain. Expert opin Emerging drugs 2005; 10(1):95-108.
8. Beyreuther B, Callizot N< Stohr T, Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy. Eur J Pharmacol 2006; 539(1-2):64-70.
9. Beyreuther BK, Freitag J, Heers C, Krebsfanger N, Scharfenecker U, Stohr T, Lacosamide : a review of preclinical properties. CNS drug Rev 2007; 13(1):21-42.
10. Bialer m, johannessen SI, Kupferberg HJ, Levy RH, Loiseau P, Perucca E. progress report on new antiepileptic drugs: a summary of the Sixth Eilat conference (EILAT VI). Epilepsy Res 2002; 51:31-71
11. Thomas Stohr, Eva Krause, Norma Selve , Lacosamide displays potent antinociceptive effects in animal models for inflammatory pain, European Journal of Pain 10 (2006) 241–249. doi: 10.1016/j.ejpain.2005.04.002
12. Hempenstall K, Rice AS. Current treatment options in neuropathic pain. Curr Opin Investig Drugs 2002;3: 441-8.
13. Bosnjak S, Jelio S, Susniar S, Lukic V. Gabapentin for relief of neuropathic pain related to anticancer treatment: A preliminary study. J Chemother 2002;14: 214-9.
14. Osikowicz M, Makuch W, Przewlocka B Mika J: Glutamate receptor ligands attenuate allodynia and hyperalgesia and potentiate morphine effects in a mouse model of neuropathic. Pain 2008; 139(1):117-26.
15. Tammy AM J.Lindsay, MD, Blakec. RodgerS J. Treating Diabetic Peripheral Neuropathic Pain. 2010.Volume 82. Number 2. page 127-155.
16. Courteix C, Eschalier A, Lavarenne J. Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain. 1993 Apr 30; 53(1):81-8.
17. Morani AS, Bodhankar SL. Neuroprotective effect of early treatment with pioglitazone and pyridoxine hydrochloride in alloxan induced diabetes in rats. Pharmacol online 2007; 2: 418-28
18. Sharma M., Katyal T, Grewal G., and Behera D. & Budhiraja R. D. Effect of antioxidants such as β-carotene, vitamin C and vitamin E on oxidative stress, thermal hyperalgesia and cold allodynia in

streptozotocin induced diabetic rats. The Internet Journal of Pharmacology. 2009 Volume 6 Number 2

19. Zafar Ahmad Malik, Nahida Tabassum, Pyare Lal Sharma. Attenuation of experimentally induced diabetic neuropathy in association with reduced oxidative-nitrosative stress by chronic administration of Momordica charantia. Advances in Bioscience and Biotechnology. 2013. 4. 356-363
20. Erichsen HK and Blackburn-Munro G. Pharmacological characterization of the spared nerve injury model of neuropathic pain. Pain 2002; 98: 151–161.
21. Amitage P, Berry G, Medical Research, Statistical method. In:AmitageP, Bery G editor, Medical research3rd ed. London:Blackwell ScientificPublications;1994,12-43.
22. Moutal Aubin, Chew Lindsey A; Yang Xiaofang, Wang Yue, Yeon Seul Ki, Telemi Edwin, Meroueh Seeneen, Park Ki Duk, Shrinivasan Raghuraman, Gilbraith Kerry B, Qu Chaoling, Xie Jennifer Y, Patwardhan Amol, Vanderah Todd W, Khanna May, Porreca Frank, Khanna Rajesh; (S)-lacosamide inhibition of CRMP2 phosphorylation reduces postoperative and neuropathic pain behaviors through distinct classes of sensory neurons identified by constellation pharmacology. 2016, 157 (7):1448-63 Pain. doi: 10.1097/j.pain.0000000000000555.
23. Fox, A., Eastwood, C., Gentry, C., Manning, D., Urban, L., 1999. Critical evaluation of the streptozotocin model of painful diabetic neuropathy in the rat. Pain 81, 307–316.
24. Malcangio, M., Tomlinson, D.R., 1998. A pharmacologic analysis of mechanical hyperalgesia in streptozotocin/diabetic rats. Pain 76, 151–157.
25. Bialer, M., Johannessen, S.I., Kupferberg, H.J., Levy, R.H., Loiseau, P., Perucca, E., 2002. Progress report on new antiepileptic drugs: a summary of the Sixth Eilat Conference (EILAT VI). Epilepsy Res. 51, 31–71.
26. Hovinga, C.A., 2003. SPM-927. IDrugs 6, 479–485.
27. Nakamura-Craig, M., Follenfant, R.L., 1995. Effect of lamotrigine in the acute and chronic hyperalgesia induced by PGE2 and in the chronic hyperalgesia in rats with streptozotocin-induced diabetes. Pain 63, 33–37.
28. Calcutt N. A. and Chaplan S. R., "Spinal pharmacology of tactile allodynia in diabetic rats," British Journal of Pharmacology, vol. 122, no. 7, pp. 1478–1482, 1997.
29. Rakieten N., Rakieten M. L., and Nadkarni M. R., "Studies on the diabetogenic action of streptozotocin (NSC-37917)," Cancer Chemotherapy Reports, vol. 29, pp. 91–98, 1963.
30. Courteix C., Eschalier A., and Lavarenne, "Streptozocin induced diabetic rats: behavioural evidence for a model of chronic pain," Pain, vol. 53, no. 1, pp. 81–88, 1993.
31. M. Meeus and J. Nijs, "Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome," Clinical Rheumatology, vol. 26, no. 4, pp. 465–473, 2007.
32. K. T. Velazques, H. Mohammad, and S. M. Swetzer, "Protein kinase in pain: involvement of multiple isoforms," Pharmacological Research, vol. 55, no. 6, pp. 578–589, 2007.
33. Bettina Beyreuther, Noelle Callizot, Thomas Stohr, Antinociceptive efficacy of lacosamide in a rat model for painful diabetic neuropathy, European Journal of Pharmacology 539 (2006) 64–70.
34. Nandita A.R., Joshi D.G., The Antihyperalgesic activity of lacosamide and amitriptyline in combination in diabetes induced neuropathy, Ijppr.Human, 2015; Vol. 4 (3): 326-362.
35. Richard L. Rauck, Aziz Shaibani, Victor Biton, Jeff Simpson, Brigitte Koch, Lacosamide in Painful Diabetic Peripheral Neuropathy A Phase 2 Double-blind Placebo-controlled Study, Clin J Pain _ Volume 23, Number 2, February 2007.
36. Bajwa SS, Kulshrestha A. Lacosamide: A novel antiepileptic and anti-nociceptive drug on the block. J Sci Soc 2014; 41:227-31.
37. Backonja MM: Use of anticonvulsants for treatment of neuropathic pain. Neurology 59: S14-S17, 2002.
38. Chandramouli J: Newer anticonvulsant drugs in neuropathic pain and bipolar disorder. J Pain Palliat Care Pharmacother 16:19-37, 2002
39. Chong MS, Libretto SE: The rationale and use of topiramate for treating neuropathic pain. Clin J Pain 19:59-68, 2003
40. Pappagallo M: Newer antiepileptic drugs: possible uses in the treatment of neuropathic pain and migraine. Clin Ther 25:2506-2538, 2003
41. Soderpalm B. Anticonvulsants: aspects of their mechanisms of action. Eur J Pain 6(Suppl A):3-9, 2002.
42. Tremont-Lukats IW, Megeff C, Backonja MM: Anticonvulsants for neuropathic pain syndromes: mechanisms of action and place in therapy. Drugs 60:1029-1052, 2000.

***Corresponding Author:**
Giram Padmaja S*
Email: **varshagiram@gmail.com**