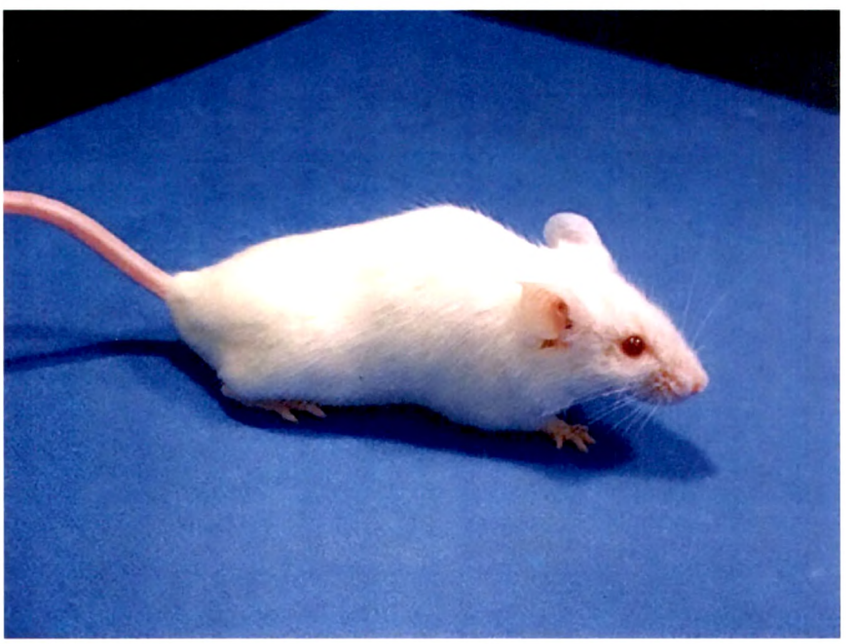
Chapter 10

Pharmacodynamic

Studies



10. 1. Introduction

Pain is part of life and related with the various disease condition such as tissue trauma, inflammatory conditions and surgical interventions that present throughout human development, from birth to death. Pain plays a crucial role for survival because it acts as a protective and alarm mechanism. According to a statement described by the International Association for the Study of Pain, pain is an unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in terms of such damage (Rocha et al., 2007).

Pain etiology is the encoding and processing of noxious stimuli by the Central Nervous System (CNS). When a noxious stimulus is passes to normal tissue, physiological nociceptive pain occurs and withdrawal reflexes are activated. Pathophysiological nociceptive pain occurs when tissue is inflamed or injured; it may appear as spontaneous pain (Schaible et al., 2004, Basbaum et al., 2002).

A laboratory measure of pain behavioral generally carried out on mice or rats. All the pain measurement methods used are especially directed towards detecting different modalities of pain through variation of the type of stimulus, its duration, intensity or localization (Franklin et al., 1989). Moreover, the nociceptive testing method can also measure the outcome with opposing alterations in pain reactivity (i.e. hypo and hyperalgesia) when testing a single treatment by different methods (Autier et al., 2000).

The tail-flick (D'Amour et al., 1941) and the hot plate test (Woolfe et al., 1944) methods are the most popular methods among the different analgesic tests using thermal nociceptive stimulation. In the hot plate test, animals are exposed only once to the heat stimulus, resulting in minimal tissue injury. The assay may be performed without any previous habituation. A hot plate method offers good reliability, reproducibility and sensitive method for detecting both hyperalgesic and analgesic responses.

Migraine is a most common, multifactorial, neurovascular disorder categorized by photophobia (visual disturbance), phonophobia (listening disturbance) repeated disabling attacks of moderate to *severe* headache with nausea, vomiting and neurologic aura symptoms (Ferrari et al., 2002). Migraine state is also considered a state of central neuronal hyper excitability in which over activity of the excitatory

amino acids. In patient with migraine, the higher concentrations of excitatory amino acids such as glutamic and aspartic acid were observed in plasma, saliva, cerebrospinal fluid. Recently it has been proposed that the throbbing pain of migraine, which is the pulsating pain aggravated by routine physical activities, is mediated by sensitization of peripheral trigeminovascular neurons, and that cutaneous allodynia (CA) of migraine, which means pain resulting from a non-noxious stimulus to normal skin, is mediated by sensitization of central trigeminovascular neurons (Malick et al., 2000).

Glutamate, an excitatory neurotransmitter, produces its action by acting on N-methyl-D-aspartate (NMDA) receptors. NMDA receptors play a key role in the nociceptive transmission within the spinal cord. Hyper activity of the excitatory amino acids (central neuronal hyper excitability) leads the migraine state, in which the higher concentrations of excitatory amino (such as glutamic and aspartic acid) were found in plasma, saliva, cerebrospinal fluid. An excitatory neurotransmitter such as glutamate, act on N-methyl-D-aspartate (NMDA) receptors that transmitted the nociceptive within the spinal cord. This was proven by the intrathecal injection of NMDA in mice induced the short duration hyperalgesia (Kitto et al., 1992, Tao et al., 2000).

10.2. Animals used

Swiss albino mice weighing between 20 to 25 g were selected for the study on the basis of randomization technique. The animal was made in groups consisted 5 animal in each group. All animal experiments conducted were approved by the Social Justice and Empowerment Committee for the purpose of control and supervision on animals and experiments, Ministry of Government of India.

10.3. Materials

Cyclobenzaprine HCl (CBZ) was obtained as a gift sample from Ranbaxy Laboratory, Gurgaon, India. N-methyl-D-aspartate (NMDA) was purchase from the Sigma Aldrich, Bangalore, India. Tizanidine HCl (TZ) was obtained as a gift sample from the Endoc Pharma, Rajkot, India.

10.4. Evaluation of N-methyl-D-aspartate (NMDA) induced central hyperalgesia in swiss albino mice for CBZ formulations

Swiss albino mice weighing between 20 to 25 g were selected for the study on the basis of randomization technique. The animal was made in groups consisted 5 animal

in each group. Hyperalgesia was induced in the swiss albino mice by the 5 µL (1.64 µg/mice) NMDA administration by intrathecal route. NMDA was administered 15 minutes before the hot plate test. The formulations (CBZ solution/LMC-CBZ NPs/ MMC-CBZ NPs/LMTC-CBZ NPs/MMTC-CBZ/LMTMC-CBZ/ MMTMC-CBZ NPs equivalent to 0.026 mg of CBZ/mice) were administered 10 µl in each nostril using micropipette (10µL to100µL) attached with low-density polyethylene tubing with internal diameter of 0.1mm at the delivery site simultaneously with the NMDA so the maximum effect of NMDA on the licking latency was seen after a few minutes of administration. However, the NMDA induced hyperalgesia was consider as a model of treatment of central sensitization. For the comparison study CBZ solution equivalent to 0.026mg of CBZ/mice administered via oral route. Hot plate method was used for the evaluation of hyperalgesia. Mice were placed on the stainless steel plate and thermostatically set the temperature up to 55° C ±0.1°C. Reaction time was measures using stopwatch before and after the treatment. Licking of the fore of hind paws was considered the end point. The mice reaction time less than 12 and more that 18 seconds before treatment were rejected from the study. An arbitrary cut off was taken 45 seconds (Ghelardini et al., 2004). Figure 10.1 shows the percent maximum possible effect (%MPE) of CBZ formulations.

10.5. Evaluation of the antinociceptive effect in swiss albino mice for TZ formulations

Swiss albino mice weighing between 20 to 25 g were selected for the study on the basis of randomization technique. The animal was made in groups consisted 5 animal in each group. The formulations (TZ solution/LMC-TZ NPs/ MMC-TZ NPs/LMTC-TZ NPs/MMTC-TZ/LMTMC-TZ/MMTMC-TZ NPs equivalent to 0.0208 mg of TZ/mice) were administered 10 µl in each nostril using micropipette (10µL to100µL) attached with low-density polyethylene tubing with internal diameter of 0.1mm at the delivery site. For the comparison study TZ solution equivalent to 0.0208 mg of TZ/mice administered via oral route. Hot plate method was used for the evaluation of antinociceptive effect. Mice were placed on the stainless steel plate and thermostatically set the temperature up to 55° C ±0.1°C. Reaction time was measured using stopwatch before and after the treatment. Licking of the fore of hind paws was considered the end point. The mice reaction time less than 12 and more that 18

seconds before treatment were rejected from the study. An arbitrary cut off was taken 45 seconds (Ghelardini et al., 2004, Langerman et al., 1995). Figure 10.2 shows the percent maximum possible effect (%MPE) of TZ formulations.

10.6. Data analysis

The results of hot plate method were evaluated by nociceptive thresholds for the hot plate test that converted to percent maximum possible effect (% MPE) according to the formula (Schreiber et al., 1999; Sawynok et al., 1999).

$$\% \text{ MPE} = \frac{\text{(Posttreatment value)- (Pretreatment value)}}{\text{(Cut off value) – (Pretreatment value)}} \text{ X } 100$$

When several groups were compared, statistical analysis was carried out using an initial one-way analysis of variance (ANOVA). P values of less than 0.05 were considered significant.

10.7. Results and discussions

10.7.1. Evaluation of N-methyl-D-aspartate (NMDA) induced central hyperalgesia in swiss albino mice for CBZ formulations

Hyper activity of the excitatory amino acids (central neuronal hyper excitability) leads the migraine state, in which the higher concentrations of excitatory amino (such as glutamic and aspartic acid) were found in plasma, saliva, cerebrospinal fluid. An excitatory neurotransmitter such as glutamate, act on N-methyl-D-aspartate (NMDA) receptors that transmitted the nociceptive within the spinal cord. This was proven by the intrathecal injection of NMDA in mice induced the short duration hyperalgesia (Kitto et al., 1992, Tao et al., 2000). Results of the hot plate method were graphically shown in the Figure 10.1. CBZ solution, CBZ loaded chitosan/thiolated chitosan/trimethyl chitosan NPs were able reverse the reduced licking latency observed by intrathecal administration of NMDA via intranasal route. CBZ loaded thiolated chitosan/trimethyl chitosan shows significantly ($p<0.01$) higher capability to reverse the NMDA-induced hyperalgesia, when administered 15 and 30 minutes before the hot plate test (Figure 10.1) than the CBZ loaded chitosan NPs and CBZ solution through the intranasal route. All the CBZ formulations have higher ability to reverse the NMDA-induced hyperalgesia via intranasal route than the oral route when

administered 15 and 30 minutes before the hot plate test. These findings may be associated with the improvement of mucoadhesion and permeability of the chitosan by thiolation and methylation. Moreover, thiolation helps in inhibiting the metabolism of CBZ by the cytochrome P450 enzymes present in the nasal cavity that reduces the breakdown of the drugs administered in the nasal cavity.

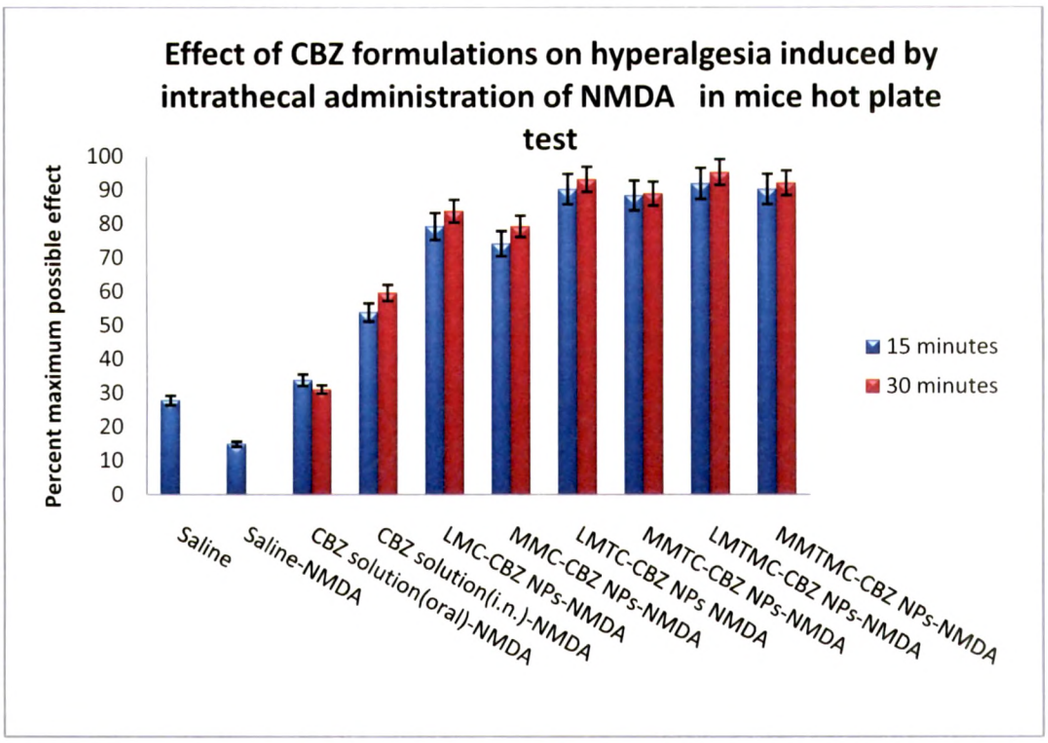


Figure 10.1: Effect of CBZ formulations on hyperalgesia induced by intrathecal administration of NMDA in mice hot plate test

10.7.2. Evaluation of the antinociceptive effect in swiss albino mice for TZ formulations

Results of the hot plate method were graphically shown in the Figure 10.2. Results shows that the antinociceptive effect of intranasal administered TZ formulations was significant higher ($p<0.05$) than the oral administration of TZ solution after 30 minutes. TZ loaded thiolated chitosan/trimethyl chitosan NPs shows significantly high antinociceptive effects than the TZ loaded chitosan NPs. These findings may be due to the high mucoadhesion and permeation capacity of trimethyl chitosan and thiolated chitosan than the chitosan. High permeation of thiolated chitosan and trimethyl chitosan was due to improvement of solubility of chitosan at physiological pH after thiolation and trimethylation respectively. Intranasal TZ solution shows significantly

lower antinociceptive effect than the NPs formulations. Theses results may be explained by the reversibly opening of the paracellular route by chitosan for hydrophilic molecules so more drug can absorbed through nasal mucosa (Langerman et al., 1995).

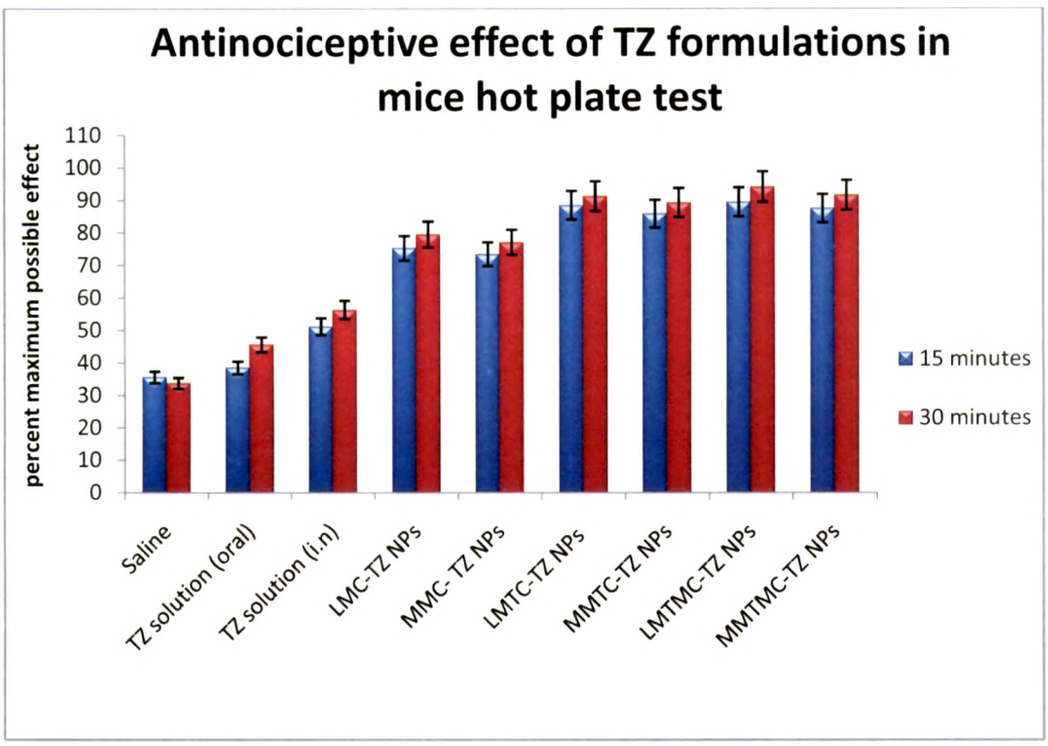


Figure 10.2: Antinociceptive Effect of TZ formulations on mice hot plate test

10.8. Conclusions

The results of present experimental show significant high antinociceptive activity of CBZ/TZ loaded thiolated chitosan and trimethyl chitosan NPs via intranasal route. CBZ loaded thiolated and trimethyl chitosan also show the antimigraine activity via intranasal route. Both the drug shows effective pain alleviation activity when administered via intranasal route and drug loaded NPs formulations may be important especially when it would be used as an alternative pain relief agent.

10.9. References

Autier, N., Gillet, J.P., Fialip, J., Eschalier, A., Coudore, F. (2000). Description of a short-term taxol-induced nociceptive neuropathy in rats. Brain Res., 887(2), 239–249.

Basbaum, A., Bushnell, C. (2002). Pain: basic mechanisms: World Congress on Pain. (10th, San Diego, CA) Pain, 1, 3–7.

D'Amour, F.E., Smith, D.L. (1941). A method for determination of pain sensation. J Pharmacol Exp Ther.,72(1), 74–79.

Ferrari, M.D., Goadsby, P.J., Roon, K.I., Lipton, R.B. (2002). Triptans (serotonin, 5-HT1B/1D agonists) in migraine: Detailed results and methods of a meta-analysis of 53 trials. Cephalalgia, 22 (8), 633-658.

Franklin, K.B.J., Abbott, F.V. (1989). Techniques for assessing the effects of drugs on nociceptive responses. In: Boulton, A.A., Baker, G.B., Greenshaw, A.J., editors. Neuromethods 13. Humana Press, New Jersey, 145–216.

Ghelardini, C., Galeotti, N., Grazioli, I., Uslenghi, C. (2004). Indomethacin, Alone and Combined with Prochlorperazine and Caffeine, but not Sumatriptan, Abolishes Peripheral and Central Sensitization in In Vivo Models of Migraine. The Journal of Pain, 5 (8), 413-419.

Kitto, K.F., Haley, J.E., Wilcox, G.L. (1992). Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neuroscience Letters, 148 (1-2), 1-5.

Langerman, L., Zakowski, M.I., Piskoun, B., Grant, G.J. (1995). Hot Plate Versus Tail Flick: Evaluation of Acute Tolerance to Continuous Morphine Infusion in the Rat Model. Journal of Pharmacological and Toxicological Methods, 34(1), 23-27.

Malick, A., Burstein, R. (2000). Peripheral and central sensitization during migraine. Funct Neurol., 15 (3), 28-35.

Rocha, A.P., Kraychete, D.C., Lemonica, L., Carvalho, L.R., Barros, G.A.M., Garcia, J.B.S., Sakata, R.K. (2007). Pain: current aspects on peripheral and central sensitization. Rev. Bras. Anestesiol., 57 (1), 94–105.

Sawynok, J., Esser, M.J., Reid, A.R. (1999). Peripheral antinociceptive actions of desipramine and fluoxetine in an inflammatory and neuropathic pain test in the rat. Pain, 82 (2), 149– 158.

Schaible, H.G., Richter, F. (2004). Patho physiology of pain. Langenbecks Arch Surg., 389 (4), 237–243.

Schreiber, S., Backer, M.M., Pick, C.G. (1999). The antinociceptive effect of venlafaxine in mice is mediated through opioid and adrenergic mechanisms. Neurosci. Lett., 273(2), 85– 88.

Tao, Y.X., Johns, R.A. (2000). Activation of cGMP-dependent protein kinase I alpha is required for N-methyl-D-aspartate- or nitric oxide-produced spinal thermal hyperalgesia. European Journal of Pharnacology, 392(3), 141-145.

Woolfe, G., McDonald, A.D. (1944). The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J Pharmacol Exp Ther.,80(3), 300–307.