ejpmr, 2020,7(4), 383-389

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

TO STUDY AND EVALUATION OF NEUROPROTECTIVE POTENTIAL OF BENIDIPINE IN FERRIC CHLORIDE INDUCED POST TRAUMATIC EPILEPSY IN MICE

Rinki*[1], Shiva Yadav*[2], Yashpal Singh*[1] and Vivek

[1]International Institute of Pharmaceutical Sciences, Sonipat.
[2]Kusum Health Care Pvt Ltd., New Delhi.

***Corresponding Author: Rinki**

International Institute of Pharmaceutical Sciences, Sonipat.

Article Received on 29/01/2020 **Article Revised on 19/02/2020** **Article Accepted on 09/03/2020**

ABSTRACT

Background:- Post-traumatic epilepsy (PTE) is a form of symptomatic epilepsy defined by the presence of recurrent seizures secondary to traumatic brain injury, presenting with variable latency of onset and course. Traumatic brain injury (TBI) greatly increases the risk for a number of mental health problems and is one of the most common causes of medically intractable epilepsy in humans. **Objective**: To study and evaluation of neuroprotective potential of benidipine in post traumatic epilepsy in mice. **Methods**: In present study, post traumatic epilepsy was induced by intracortical injection of ferric chloride. Mice were anesthetized with intraperitoneal injection of ketamine (80mg/kg) and xylazine (20 mg/kg). A midline sagittal incision of 1cm was made on scalp. Bregma and lambda were located and the centre point between the two was marked. The skull was exposed and a burr hole was made in the location over the left sensorimotor cortex, 1mm posterior and 2mm lateral to bregma. Ferric chloride solution (100mM, 5µl) was injected 2mm below skull at rate of 1µl/min through a hamilton syringe. **Result**:- In present study, PTE was induced by ferric chloride induced model of post-traumatic epilepsy & benidipine 9mg/kg; p.o. showed most neuroprotective potential improving behavioral parameters (open field test, rota rod test, actophotometer test and anhedonia test) and oxidative damage. **Conclusion**: This study demonstrated the neuroprotective potential of benidipine in post traumatic epilepsy in mice.

KEYWORD: Epilepsy, Post-traumatic epilepsy (PTE), Traumatic brain injury.

INTRODUCTION

Post-traumatic epilepsy is a form of symptomatic epilepsy defined by the presence of recurrent secondary to traumatic brain injury.[1] Post-traumatic epilepsy further aggravates pathological injury of brain tissue and neurobiochemical changes, increases risk of death, and results in huge mental and employment pressure in addition to economic burden. It is one of the most common neurological disorders with individual, familial and social economic impacts. It has found that post traumatic epilepsy accounts for 10-20% of symptomatic epilepsy in the general population.[2] It has been observed that the presently available 25% drugs are unable to alleviate the symptoms of seizures.[3] Therefore there is need to investigate for agents that are highly efficacious as well as safe in term of drug related toxicity.

There are multiple theories that have been developed to explain the mechanisms behind what causes chronic seizures after a head injury. Among these are the formation of damaging free radicals by blood in the parenchyma of the brain, increases in excitatory activity following injury, and changes in the inhibitory functions of the brain. As the mechanisms behind the development of post-traumatic epilepsy are discovered, therapies may be developed that can prevent epilepsy after head trauma has occurred. Several animal models have also been developed to explore the possibilities of different types of head injury from concussions to penetrating head injury. Since parenchymal blood is associated with an increased risk in developing epilepsy, possible mechanism involving this type of insult have been explored. Haemoglobin was studied as an agent involved in epileptogenensis. Once there is bleeding into brain tissue, red blood cells are lysed with a subsequent release in haemoglobin that is broken down into hemin and iron. Both breakdown products have been shown to have physiological effects on synaptic transmission that may lead to epileptogenesis.[4] There is an animal model where epilepsy is induced by injecting ferric chloride into the brain of mice. The epileptogenic effect of iron is thought to be related to the formation of free radicals that cause direct injury to neuronal membranes and cell death. Iron injected into the brain has also been found to affect the release and metabolism of the excitatory neurotransmitter glutamate.

Glutamate is the major excitatory neurotransmitter in the central nervous system(CNS) and acts on ionotropic and metabotropic glutamate receptors located at the presynaptic terminal and in the postsynaptic membrane at synapses in the brain and spinal cord. GABAergic inhibition appears to be primarily due to interneuronal loss. The neurotransmitter GABA activates a Cl^- selective ligand-gated ion channel ($GABA_A$) in the mammalian CNS and produces neural inhibition. Diverse therapeutic and pharmacological agents can modulate $GABA_A$ receptor function. Thus, the $GABA_A$ receptor remains a major therapeutic target for a number of indications. Calcium is thought to be the main ion involved in excitotoxicity. Excitatory glutamate receptors are important in the influx of calcium into the cell. L type Ca^{2+} channel blockers such as the 1,4-dihydropyridines, phenylalkylamines and benzothiazepines are primarily used for the treatment of cardiovascular diseases. More recently, it has been suggested that it may be useful in other pathological states including seizures and central ischemic disorders.

Benidipine is a third-generation dihydropyridine (DHP) type of calcium channel antagonist. It is triple L, T and N-type calcium channel blocker. Benidipine has slow onset of action and long lasting property.[5] There was no prominent study which shows the role of benidipine in post traumatic epilepsy. Therefore, the present study was designed to evaluate the pharmacological potential of benidipine in post traumatic epilepsy. In the present study, the behavioural and biochemical parameters were performed. Behavioural studies include various parameters. After 1 h of benidipine administration, post traumatic epilepsy was induced through intracortical injection of the ferric chloride (5µl,100mM). Animals were observed after recovery from surgery, open field test for explotation, rota rod test for motor coordination activity, anhedonia test and actophotometer for locomotor activity. In biochemical estimation, the effect of benidipine on oxidative biomarkers were explored by measuring protein, malonyldialdehyde, reduced glutathione, catalase and nitrite levels.

There was significant increase in the level of nitrite and malonyldialdehyde and decreased in the level of protein, glutathione and catalase was significantly increased in post traumatic epileptic brain regions with benidipine treatment. There was also dose dependent and significant increase in fall off time, increase in number of squares crossed, increased water consumption and increase in the locomotor activity. Benidipine was evaluated for its neuroprotective potential through its antioxidant property.

Thus, this study was designed to investigate the neuroprotective potential of benidipine in ferric chloride induced post traumatic epilepsy. Benidipine was administered at three different doses (3mg/kg, 6mg/kg and 9mg/kg). It showed dose dependent and significant effect on motor coordination, exploration, taste preference, locomotor activity and oxidative stress. The maximum results were observed by benidipine 9mg/kg; p.o. treated groups.

MATERIAL AND METHOD

Animals

Swiss albino mice with weight 20-30 g were used in the study. They were procured from diseased free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, after the approval from Institutional Animal Ethical Committee (IAEC) of Maharshi Dayanand University, Rohtak.

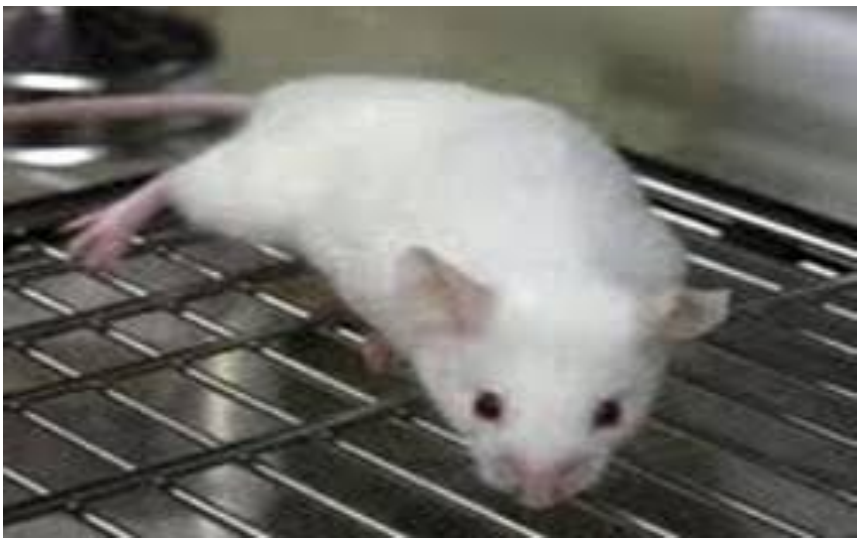

Fig. 1: Swiss albino mice.

Ferric Chloride induced model of post traumatic epilepsy model

In present study, post traumatic epilepsy was induced by intracortical injection of ferric chloride (Loba Chemie Pvt. Ltd., Mumbai). Mice were anesthetized with intraperitoneal injection of ketamine (80mg/kg) and xylazine (20 mg/kg). A midline sagittal incision of 1cm was made on scalp. Bregma and lambda were located

and the centre point between the two was marked. The skull was exposed and a burr hole was made in the location over the left sensorimotor cortex, 1mm posterior and 2mm lateral to bregma. Ferric chloride solution (100mM, 5µl) was injected 2mm below skull at rate of 1µl/min through a hamilton syringe. The needle remained for 1 min after injection.[6] Ferric chloride was freshly prepared.

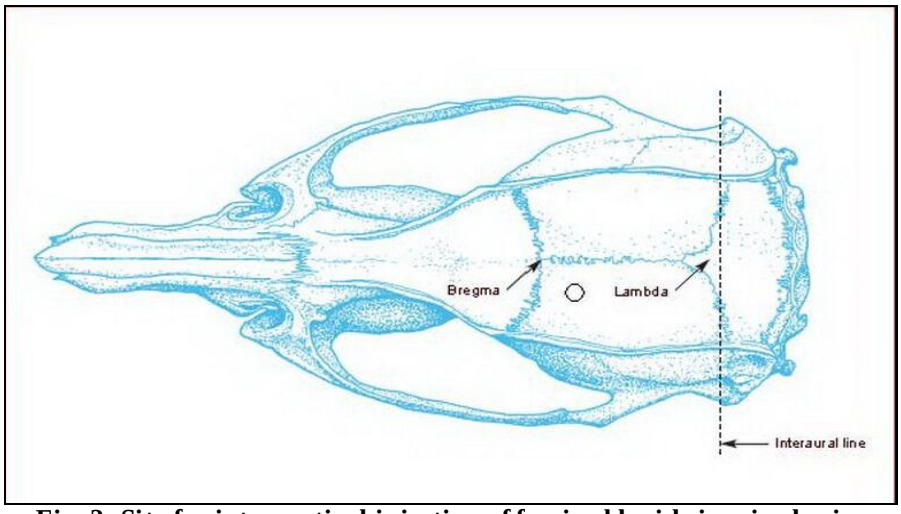

Fig. 2: Site for intracortical injection of ferric chloride in mice brain.

Experimental plan
Group 1: Control group
Group 2: $FeCl_3$ (5µl, i.c.v) + Vehicle (normal saline)
Group 3: $FeCl_3$ (5µl, i.c.v.) + Benidipine (3mg/kg, p.o.)
Group 4: $FeCl_3$ (5µl, i.c.v.) + Benidipine (6mg/kg, p.o.)
Group 5: $FeCl_3$ (5µl, i.c.v.) + Benidipine (9mg/kg, p.o.)

Treatment schedule
In group 1, no treatment was given to mice. In group 2, 3, 4 and 5, after anaesthesia $FeCl_3$ (5µl, 100mM, i.c.v.) (Loba Chemie Pvt. Ltd. Mumbai, India) will be administered to mice. In group, 3, 4 and 5 prior to 1h of $FeCl^{3+}$ benidipine (3, 6 and 9 mg/kg, p.o.) was given (Caritac, Sun Pharmaceuticals Pvt. Ltd., Mumbai, India) in group 3, 4 and 5 respectively. $FeCl_3$ was administered only on day1 and drug benidipine was given daily till day 21 in group 3, 4 and 5. After recovery from anaesthesia, behavioural parameters will be observed on day1, day 7, day 14 and day 21 and after 24 h, on day 21 biochemical parameters will be observed.

Behavioural parameters: After 1h of administration of drug, behavioural parameters will be observed.

1) Open Field Test
The apparatus, made of white wood, had a floor of 100 X 100 cm divided by red lines into 25 squares of 20 X 20 cm. The walls, 50-cm high, will be also painted in white. The test room will be illuminated at the same intensity as the colony room. Each mouse will be placed in the centre of the open field, and its behaviour will be observed for 5 min. The parameters evaluated will be the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures will be referred to as total, peripheral, and central locomotion, respectively.

Fig. 3: Open field.

The ratio of central locomotion to total locomotion (CL/TL) will calculated. The numbers of leanings (one or two paws in contact with the wall), rearings (the mouse standing on its two hind paws without touching the walls), groomings (face cleaning, paw licking, fur licking, head scraping, and rubbing), and defecations will be also recorded. At the end of each test, the whole area will be cleaned with a wet sponge and a dry paper towel.[7]

2) Rota Rod Test

The test will be used in study to assess motor coordination in mice. Mice will be trained to remain on a rota-rod with slowly revolving rods of 5 cm diameter at 16 rpm for 180 s. Mice that will be able to remain on the rod for 180 s or longer will be selected and divided into four groups of six mice per group. The animals will be placed individually on the rod. If an animal failed more than once to remain on the rod for 3 min, the test will be considered positive, meaning there will be a lack of motor coordination.[8]

Fig. 4: Rota rod.

3) Anhedonia test

Anhedonia test is based on the taste preference of rodents towards sweets. Taste preference was examined using saccharin solution consumption test. The mice cage was supplied with 100ml graduated bottles, one filled with water and another filled with 0.1% saccharin solution. Taste preference was expressed as percent of the volume of saccharin solution of a total volume of fluid (saccharin plus water) consumed over 24 h.[9]

4) Actophotometer test

Actophotometer test was used to check the locomotion activity of rodents. The movement (i.e. the number of light-beam crossing) of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. Each mice was placed individually for the 10 min. and basal activity score was obtained.

Fig. 5: Actophotometer apparatus.

Biochemical Parameters

Biochemical analysis- Biochemical estimations in the brain homogenate were carried out 24h after completion of all the behavioural assessments. Brain homogenate preparation- Animals were sacrificed by decapitation and the brains were quickly removed and washed with ice-cold sterile saline (0.9%). The whole brain samples were then homogenized with ice-cold 0.1M phosphate buffer (pH 7.4) 10 times (w/v). The homogenate was centrifuged at 2500 x g (4C) for 15min. to remove cellular debris and aliquots of supernatant were separated and used for biochemical estimations.

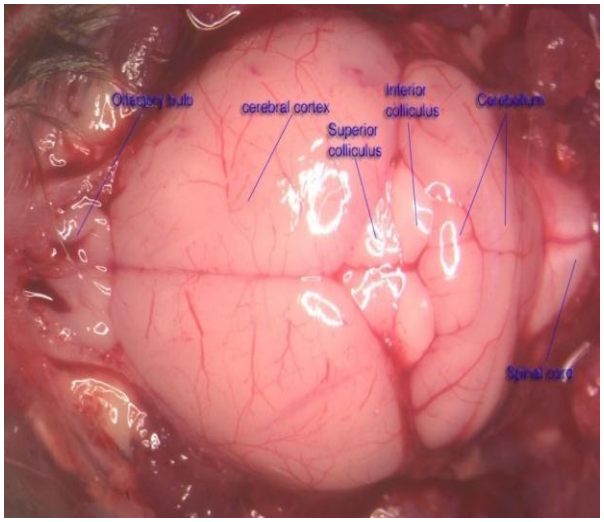

Fig. 6: Mice brain.

RESULTS

The effect of benidipine on different parameters was explained below:

Effect of benidipine on behaviour of post traumatic epileptic mice

Effect of benidipine on behaviour was evaluated by open field test, rota rod test, actophotometer test, and anhedonia test.

a) Effect of benidipine on exploratory behaviour in mice using open field test

Post traumatic epilepsy has significantly impaired the exploratory behaviour in mice which was evaluated using open field test by the parameters including number of squares crossed and rearing.

1. Number of square crossed: When compared the vehicle treated group (day 1, 7, 14, 21) (3± 0.448, 6.5± 0.766, 6.833 ±0.875, 8.166 ± 0.603) dose dependent and significant increase in number of square crossed was found in benidipine treated group i.e. benidipine 3mg/kg (4.833 ± 0.40295, 8 ± 0.448, 8.333 ± 0.496, 10.166 ± 0.705), benidipine 6 mg/kg (7.666 ± 0.496, 10.166 ± 0.705, 12.166 ± 0.705, 12.667 ± 0.559) and benidipine 9mg/kg (10 ± 0.579, 12.833 ± 0.308, 15 ± 0.366, 15.333 ± 0.334). (Fig.)

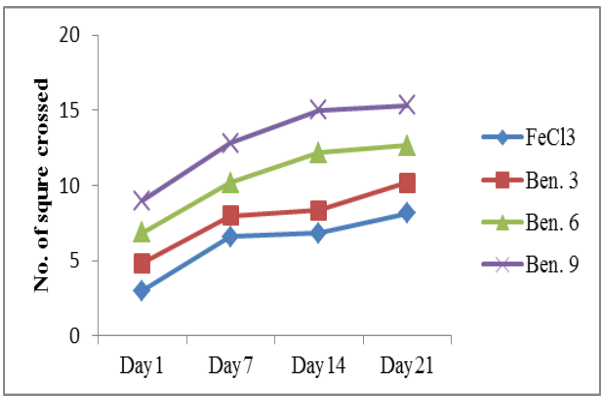

Fig. 7: Effect of benidipine on number of square crossed in ferric chloride induced post traumatic epilepsy in mice.

The figure showing 4 groups and n=6 in each groups. Values were expressed as Mean±SEM. Data was analysed by repeated measures two way ANOVA followed by Tukey test. The F value was found to be F(12,100) = 3.111. **Ben.3, Ben.6, Ben. 9:** Benidipine 3, 6 and 9 mg/kg, p.o., respectively.

b) Effect of benidipine on rota rod test in post-traumatic epileptic mice

In iron induced post traumatic epileptic mice, rota rod test for motor co-ordination and muscle relaxant property was evaluated by fall off time(in sec.). When compared the vehicle treated group (day 1, 7, 14, 21) (3.5±0.429, 5±0.969, 6±0.897, 9.166± 0.603) dose dependent and significant increase in number of square crossed was found in benidipine treated group i.e. benidipine 3mg/kg (6.333 ± 0.496, 9.5± 1.208, 8.666 ± 0.846, 12± 0.448), benidipine 6 mg/kg (8.666 ± 0.496, 10.5±1.180, 11.166± 0.603, 15± 0.518) and benidipine 9mg/kg (10.666± 0.334, 14.166± 1.199, 18± 1.017, 25± 1.429). (Fig.)

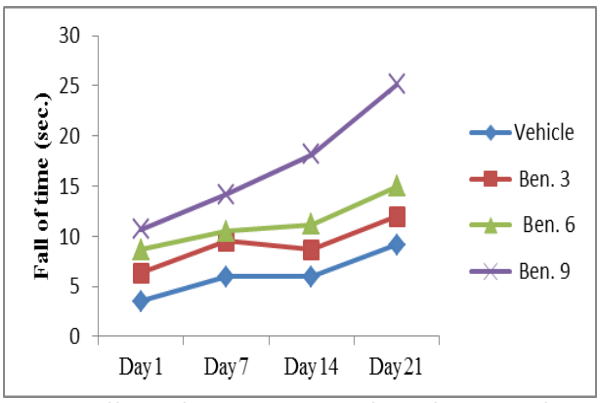

Fig. 8: Effect of benidipine on fall of time in ferric chloride induced post traumatic epilepsy in mice.

The figure showing 4 groups and n=6 in each groups. Values were expressed as Mean±SEM. Data was analysed by repeated measures two way ANOVA followed by Tukey test. The F value was found to be F (9,80) = 5.353. **Ben.3, Ben.6, Ben. 9:** Benidipine 3, 6 and 9 mg/kg, p.o., respectively.

c) Effect of benidipine on actophotometer test in post-traumatic epileptic mice

In iron induced post traumatic epileptic mice, actophotometer test used for evaluating the locomotor activity. When compared the vehicle treated group (day14, 21) (55±1.719, 52.333±1.058) dose dependent and significant increase in locomotor activity was found in benidipine treated group i.e. benidipine 3mg/kg (82.5± 3.161, 93.666± 1.208), benidipine 6 mg/kg (117.333± 5.316, 125.333±5.422) and benidipine 9mg/kg (142.333± 3.644, 151.833± 3.125). (Fig.)

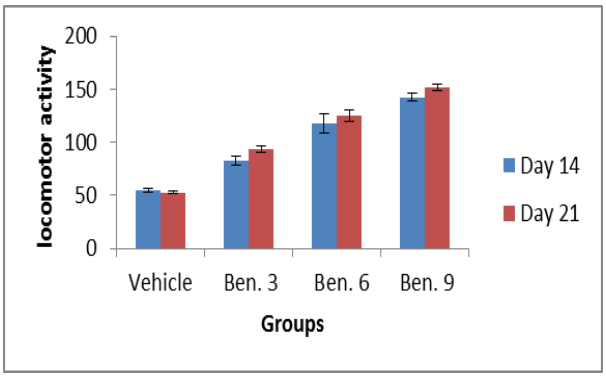

Fig. 9: Effect of benidipine on locomotor activity in ferric chloride induced post traumatic epilepsy in mice.

The figure showing 4 groups and n=6 in each groups. Values were expressed as Mean±SEM. Data was analysed by repeated measures two way ANOVA

followed by Tukey test. The F value was found to be F(4,50) = 0.6196. **Ben.3, Ben.6, Ben. 9:** Benidipine 3, 6 and 9 mg/kg, p.o., respectively.

d) Effect of benidipine on anhedonia test post traumatic epileptic mice

In iron induced post traumatic epileptic mice using anhedonia (taste preference) test property evaluate consumption of tap water and saccharin (0.1%) water in ml. Vehicle and drug treated both animals prior to preferred saccharin solution over tap water. When compared to vehicle treated group (day1, 7, 14, 21) (5, 6.5, 8 and 9ml) a dose dependent and significant increase in consumption of tap water and saccharin(0.1%) water (taste preference) property increase and total water consumption was found on day 1, 7, 14, 21 in benidipine 3mg/kg (8, 11, 12 and 13 ml), benidipine 6mg/kg (11, 16, 20 and 22 ml) and benidipine 9mg/kg (15.5, 20, 27 and 29 ml) group.

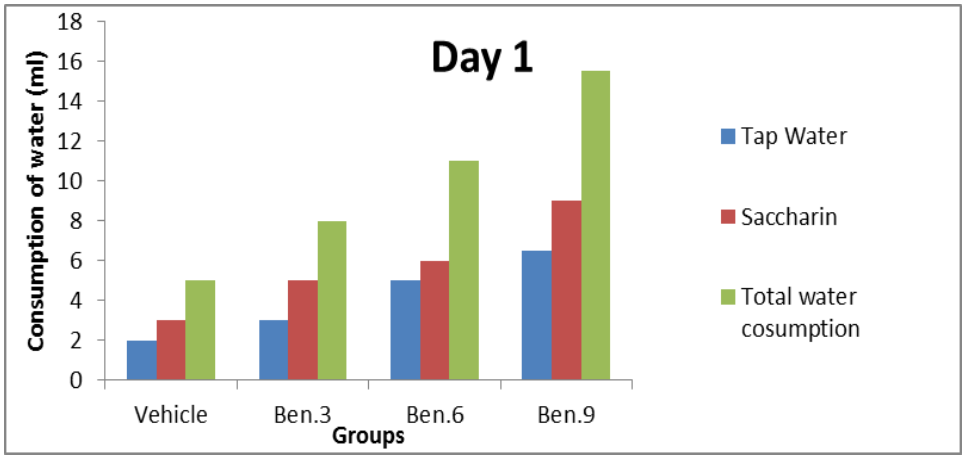

Fig. 10: Effect of benidipine on anhedonia test in ferric chloride induced epileptic mice on day 1.

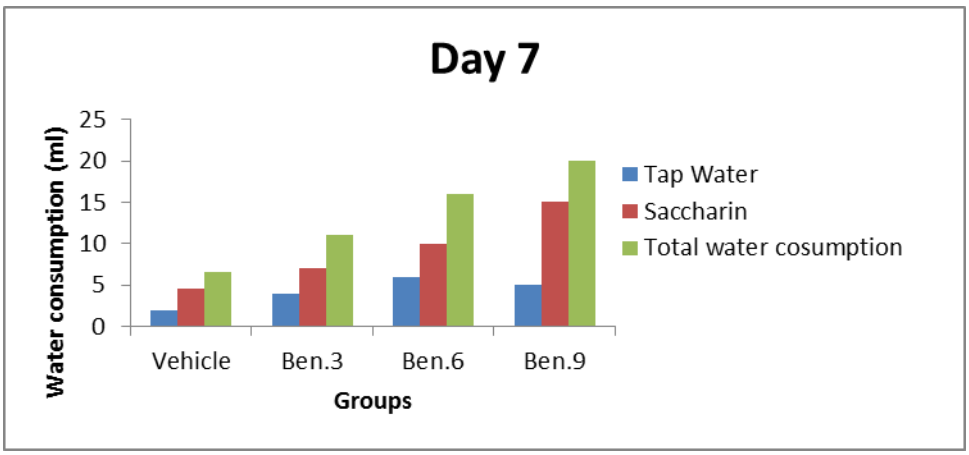

Fig. 11: Effect of benidipine on anhedonia test in ferric chloride induced epileptic mice on day 7.

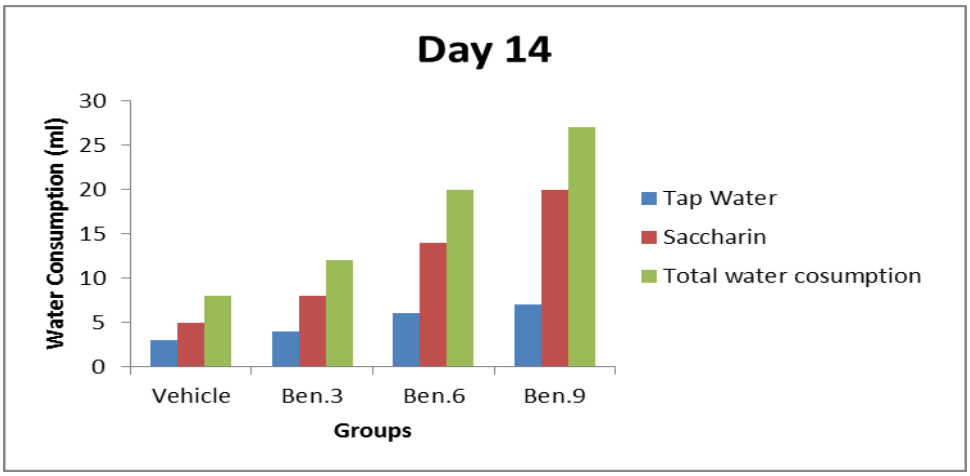

Fig. 12: Effect of benidipine on anhedonia test in ferric chloride induced epileptic mice on day 14.

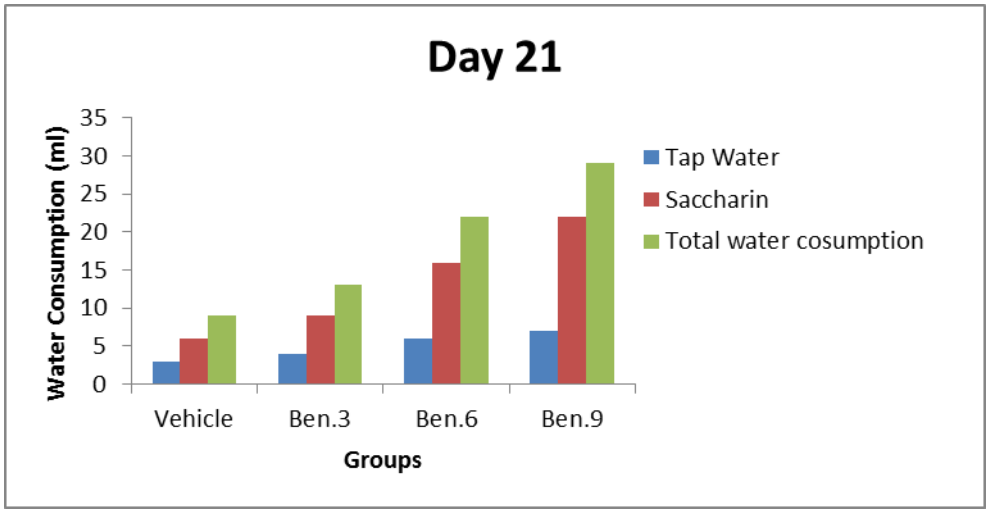

Fig. 13: Effect of benidipine on anhedonia test in ferric chloride induced epileptic mice on day 21.

This figure showing 4 groups and n=6 in each group. Values were expressed as mean. Its shows total water consumption increases in drug groups as compare to vehicle group in **Ben.3, Ben.6, Ben. 9:** Benidipine 3, 6 and 9 mg/kg, p.o., respectively.

SUMMARY

In present study, benidipine shows its action on many neurotransmitters but major interest is focused on calcium channel blocking property. Benidipine is a third-generation dihydropyridine (DHP) type of calcium channel antagonist. It is triple L, T and N-type calcium channel blocker. Benidipine has slow onset of action and long lasting property. It also shows antioxidant effect by reducing oxidative stress. There was no prominent study which shows the role of benidipine in post-traumatic epilepsy. Therefore, the present study was designed to evaluate the pharmacological potential of benidipine in post-traumatic epilepsy.

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