IJPSR (2022), Volume 13, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March 2022; received in revised form, 25 April 2022; accepted 14 October 2022; published 01 November 2022

CELECOXIB, A SELECTIVE CYCLOOXYGENASE-2 INHIBITOR, IS ANTICONVULSIVE IN MES INDUCED SEIZURES BUT PROCONVULSIVE IN PTZ INDUCED SEIZURES IN MICE: A POSSIBLE ROLE OF EXCITATORY AMINO ACID IN THIS DIFFERENTIAL EFFECT

Raina Jain [1], Ajay Upadhyaya [2] and Ashish Jain [*3]

Department of Pharmacology [1], Department of Pulmonary Medicine [2], ABV Government Medical College, Vidisha - 464001, Madhya Pradesh, India.
Department of Biochemistry [3], PCMS & RC, Bhopal - 462037, Madhya Pradesh, India.

Keywords:

Epilepsy, Inflammation, COX –2 inhibitors, Celecoxib, MES, PTZ, THLE, Clonus, GTC, Absence seizures

Correspondence to Author:
Dr. Ashish Jain

Associate Professor,
Department of Biochemistry,
PCMS & RC, Bhopal - 462037,
Madhya Pradesh, India.

E-mail: drashisjain@gmail.com

ABSTRACT: Epilepsy is an achronic neurological disorder characterized by recurrent seizures. There is a positive feedback cycle between epileptogenesis and brain inflammation. It has been proved that COX 2 is expressed constitutively in certain parts of the CNS and is further induced as a result of seizurogenic activity. Of the various experimental models of epilepsy, one is the maximal electroshock (MES) model, which is highly predictive of AED against GTCS. Other is the pentylenetetrazole (PTZ) model, which is useful in the AEDs activity against absence seizures. The present study was done to evaluate the antiepileptic effect of selective COX 2 inhibitor celecoxib in MES and PTZ models in mice and to see the differential effect of celecoxib in electrically chemically induced seizures. In the first part of the study, Celecoxib (2.5mg/kg, 5mg/kg & 10mg/kg) was injected intraperitoneally in albino mice. MES was induced, and durations of various phases were noted. Duration of Tonic hind limb extension was taken as an index of antiepileptic activity. The results indicated that celecoxibin, the dose of 10mg/kgi.p. Significantly reduced the duration of THLE. In the second part, celecoxib was injected in (10mg/kg & 20 mg/kg i.p.), in PTZ induced model, and the result displayed the proconvulsive activity in the PTZ seizure model. *i.e.*, decrease in the latency to preclonic jerk and increase in the duration of clonus. This suggested the role of inflammation and COX2 isoenzyme in the pathophysiology of epilepsy and the clinical application of COX 2 inhibitors as a future therapeutic strategy for GTC seizures management.

INTRODUCTION: Epilepsy, defined by the sporadic occurrence of spontaneous recurrent seizures (SRS) is often accompanied by inflammation of brain.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(11).4782-87

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(11).4782-87

Pronounced increase in the expression of key inflammatory mediators (*e.g.*, Interleukin – 1 β, TNF α, COX – 2 & C-X-C motif chemokine 10) after seizures may cause secondary damage in the brain & increase the likelihood of repetitive seizures [1].

Cytokine and prostaglandins are well-known mediators, and their biosynthesis is enhanced following seizures [2]. The key role of inflammatory mediators in relation to epilepsy has been clarified over the last decade. Numerous studies have shown

PGs as potential mediators of epilepsy. Studies have shown the protective effect of aspirin in MES and PTZ models of epilepsy in a dose-dependent manner and potentiation of the anticonvulsant effect of Lamotrigene with Aspirin [3, 4]. COX-2 enzyme has been shown to be upregulated by convulsive nerve activity. In some previous studies, non-selective COX inhibitors (Mefenemic acid, Melclofenemic acid, Acetaminophen) have shown to delay the onset of PTZ-induced convulsions [5, 6]. As weal ready know, the COX enzyme has two isomers, COX-1, which is widely distributed in various cell types, while COX-2 is constitutively expressed in inflammation sites. COX-2 is mainly expressed in Glutaminergic neurons, particularly in the hippocampus and cerebral cortex, the areas that demonstrate prominentrolein seizures, suggesting that COX-2 plays a critical role in convulsive states [7].

Most current AEDs act on ion channels that directly control neuron excitability. These medications have at least two major problems. First, even with optimal AED therapy, 20–30% of the patient has poor seizure control and become intractable. Second, these medications act as general CNS depressants and must be taken chronically for seizures up press ions, they also have marked inhibitory effects on cognitive development. Therefore the development of a new AED that can modulate seizures through other mechanisms is required for refractory epilepsy treatment. With the evidence of the protective effects of non-selective COX inhibitors in MES and PTZ seizures and to spare the housekeeping functions of COX-1enzyme, the present study has been planned to evaluate the anticonvulsant effect of selective COX -2 inhibitor, celecoxib, in MES and PTZ induced seizures in mice and to see its differential effect on electrically and chemically induced seizure.

MATERIAL:

Animals: Adult, healthy swiss albino mice, aged 6-8 weeks of either sex, weighing 20 – 30 g were used. They were housed under standard laboratory conditions [controlled temperature (around 22±2°C) and humidity (50%) colony room] for one week before experiments were started and were kept in groups of 3-4 in per polypropylene cages. Animals were allowed a standardized diet and water *ad libitum*, except for the period of experimentation. Techno-electro convulsometer. Stop-watch, Drugs and Chemicals Celecoxib (Cadilla pharma, Ahmedabad 0): the fresh solution was in 0.9% saline, with the dispersion of 2-3 drops of Tween 80.

Tween 80 (Himedia, Mumbai): 2-3 drops of tween 80 were dispersed in the solution of COX-2 inhibitor in 0.9% saline.

Pentylene Tetrazole (Hi media, Mumbai): stored at 0-4°C, dissolved in distilled water just before use.

METHOD: The present study was undertaken to evaluate the anti-epileptic activity of selective COX-2 inhibitor, Celecoxib, and its differential effect on both electrically (MES) and chemically (PTZ) induced seizures. In the first part of the study, the animals were grouped in three groups, each comprising 10 animals. MES seizures were induced by a Techno-electro convulsometer (50mAmp, 0.1 sec duration) through ear electrodes and durations of various phases of MES (TF, THLE, CC, and PTD) were noted. In the first phase, the animals were treated with celecoxib (2.5mg/kg i.p.). MES induced after 40 min. of administration of the drug, and various phases were noted. The animals were treated with Celecoxib in the second phase (5mg/kg i.p.). MES induced after 40 min. of administration of the drug, and various phases were noted. In the third phase, the animals were treated with celecoxib (10mg/kg i.p.). MES induced after 40 min. of drug administration and various phases were noted**.** Each study was conducted with a control group treated with distilled water. The abolition or reduction of Tonic hindlimb extension (THLE) was considered the antiepileptic activity index. In the second part of the study, the animals were divided into two groups, each comprising 10 animals. In the first phase, PTZ, in the dose of 70mg/kg ip was administered, and the duration of onset to pre-clonic seizures and duration of clonus were recorded. In the second & third phases, the animals were treated with celecoxib, 10mg/kg ip & 20mg/kg ip, respectively. Drugs were injected 40 min before inducing seizure by PTZ, and onset to preclonic seizures and duration of clonus were recorded. The results were statistically analyzed by

paired student's tests. *P-value* <0.05 were considered statistically significant

RESULTS:

MES Induced Seizures: The results of the present study show that Celecoxib in the doses of 2.5mg/kg ip and 5mg/kg ip, failed to produce any significant reduction in the duration of any phase of MES induced seizures (P>0.05) **Table 1-4, Fig. 1**. On increasing the dose of celecoxib to 10mg/kg ip, a significant decrease in the duration of tonic hind limb extension (index of antiepileptic activity) (P<0.001) **Table 2**, **Fig. 1** was found, without any change in any other phases of MES challenge (P>0.05) **Table 1-4, Fig. 1.**

TABLE 1: EFFECT OF COX-2 ON DURATION OF TONIC FLEXION (TF) IN MES INDUCED MICE

S. no.	Drugs / Dose	Tonic Flexion (seconds)	
1	Control	3.4±1.08	
2	Celecoxib (2.5mg/kgi.p.)	3.4±0.87	A_1(*)
3	Celecoxib (5mg/kgi.p.)	3.2±1.03	A_2(*)
4	Celecoxib (10mg/kgi.p.)	3.3±1.10	A_3(*)

TABLE 2: EFFECT OF COX-2 ON DURATION OF TONIC HIND LIMB EXTENSION (THLE) IN MES INDUCED MICE

S. no.	Drugs /Dose	Tonic Hind Limb Extension (seconds)	
1	Control	13.6±1.26	
2	Celecoxib (2.5mg/kgi.p.)	13.4±1.10	A_1(*)
3	Celecoxib (5mg/kgi.p.)	13.4±1.03	A_2(*)
4	Celecoxib (10mg/kgi.p.)	7.0±0.64	A_3(****)

TABLE 3: EFFECT OF COX-2 ON DURATION OF CLONIC CONVULSIONS (CC) IN MES INDUCED MICE

S. no.	Drugs / Dose	Clonic Convulsions (seconds)	
1	Control	8.6±1.17	
2	Celecoxib (2.5mg/kgi.p.)	8.4±1.10	A_1(*)
3	Celecoxib (5mg/kgi.p.)	8.5±0.87	A_2(*)
4	Celecoxib (10mg/kgi.p.)	8.5±0.99	A_3(*)

TABLE 4: EFFECT OF COX-2 ON DURATION OF POST TETANIC DEPRESSION (PTD) IN MES INDUCED SEIZURE IN MICE

S. no.	Drugs /Dose	Post Tetanic Depression (seconds)	
1	Control	6.0±0.94	
2	Celecoxib (2.5mg/kgi.p.)	6.0±0.94	A_1(*)
3	Celecoxib (5mg/kgi.p.)	5.7±0.82	A_2(*)
4	Celecoxib (10mg/kgi.p.)	6.0±0.94	A_3(*)

Value sare given mean ± SD A_1–control v/s celecoxib (2.5mg/kg) A_2 – control v/s celecoxib (5 mg/kg) A_3–controlv/scelecoxib (10mg/kg) (*)–P>0.05, (**)–P<0.05, (***)–P<0.01, (****)–P<0.001 (Paired students t test).

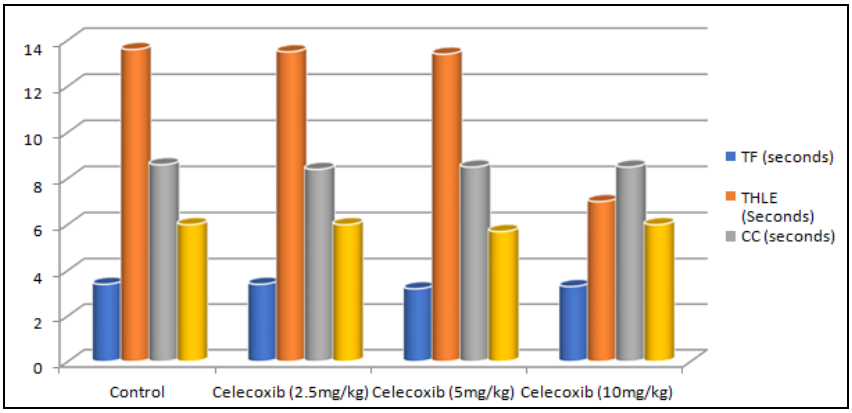

FIG. 1: EFFECT OF CELECOXIB ON DURATION OF TF, THLE, CC, PTD ON MES INDUCED SEIZURES IN MICE

PTZ Induced Seizure: Celecoxib was administered in the dose of 10mg/kg ip and 20mg/kg ip. The animals were observed for about 30 min. The latency to the onset of seizures was significantly decreased (P<0.001) **Table 4, Fig. 2** and the duration of clonus was increased **Table 5, Fig. 2** (P< 0.001)

TABLE 5: EFFECT OF COX-2 ON ONSET OF PRE CLONIC SEIZURES IN PTZ INDUCED SEIZURES IN MICE

S. no.	Drugs /Dose	Onset to pre clonic seizures (seconds)	
1	Control	71.5±3.74	
2	Celecoxib (10mg/kgi.p.)	53.7±2.89	B_1(****)
3	Celecoxib (20mg/kgi.p.)	52.3±2.20	B_2(****)

TABLE 6: EFFECT OF COX-2 ON DURATION OF CLONUS IN PTZ INDUCED SEIZURES IN MICE

S. no.	Drugs / Dose	Duration of clonus (seconds)	
1	Control	30.0±2.40	
2	Celecoxib (10mg/kgi.p.)	68.1±2.90	B_1(****)
3	Celecoxib (20mg/kgi.p.)	68.2±2.80	B_2(****)

Value sare given mean ±SD B_1– control /scelecoxib(10mg/kg) B_2– control /scelecoxib(20mg/kg) (*)–P>0.05,(**) P<0.05,(***)–P<0.01,(****) P<0.001(Paired students t test).

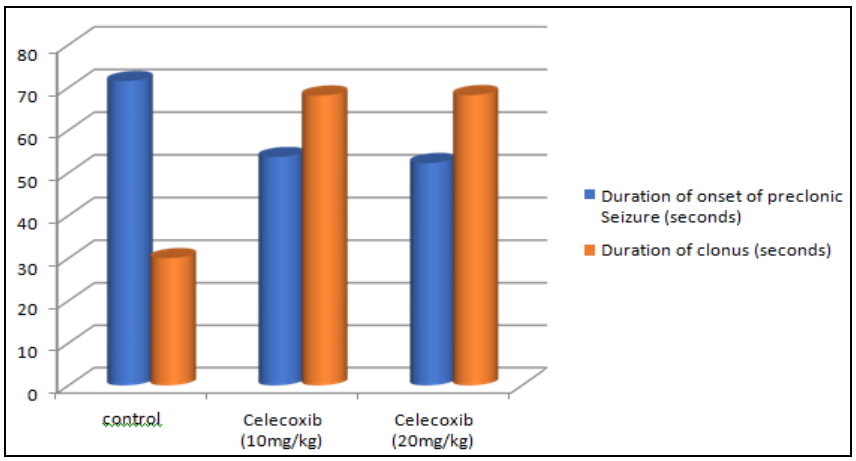

FIG. 2: EFFECT OF CELECOXIB ON DURATION OF ONSET OF PRECLONIC SEIZURES AND CLONUS ON PTZ INDUCED SEIZURES IN MICE

DISCUSSION: Results of the present study correlates with the significant antiepileptic activity of selective COX-2 inhibitor, celecoxib, in MES induced seizures. COX-2 is responsible for the pathological production of prostaglandins in response to a variety of stimuli from proinflammatory factors (*e.g.*, cytokines and endotoxins), seizure activity, brain injury to the activation of NMDA receptors (main receptors for the excitatory neurotransmitter, L- glutamate) [8]. COX – 2 proteins significantly increase in the hippocampi of genetically seizure susceptible E1 mice 9. The role of inflammation in the excitotoxicity induced by overstimulation of glutamate receptors using kainic acid and neuronal cell death, especially in CA1 and CA 3 areas of hippocampus has been investigated, and it has been hypothesized that inhibiting neuroinflammation after kainic acid injection will produce less inflammation, less cell loss and enhancement of cognitive function or both. In previous studies, brain-derived neurotropic factor levels were examined, and results confirmed the above hypothesis that kainic acid injected rats treated with a selective COX-2 inhibitor (after kainic acid injection) performed better in the spatial and non-spatial tasks. There was not any improvement if celecoxib was given before kainic acid treatment. This underlines the importance of the production of PGs at the beginning of inflammation [10]. Some researchers have studied the anticonvulsant action of pre-treatment of celecoxib in electroshock induced convulsions and found that celecoxib in dose-dependent manner increases the (a) CC50 (threshold current inducing THLE in 50% of animals; (b) Percent protection of animals [11]. Although Celecoxib suppresses epileptogenesis, this inhibitor may be more effective for post-seizure inflammation than for the prevention of seizure incidence [12]. Possible dual roles might partly explain these conflicting results for COX-2, which has been shown to play early neuroprotective and late neurotoxic roles following seizures [13]. Thus, limiting COX-2 inhibitor administration may be crucial when treating epilepsy. The bifunctional aspects of COX-2 in

epileptogenic can be explained by the diversity of PGs. PGD2 & PGF2α exhibit anticonvulsive functions; hence PGD2 synthetase knockout mice showed more severe seizures after PTZ treatment [14,] and the intracisternal administration of PGF2α after KA treatment reduced the seizure score and mortality [15]. On the other hand, PGE2 mainly functions as a promoter of epileptogenesis, and the administration of PGE2receptor (EP) antagonists reduces seizure severity and neuronal injury following pilocarpine or PTZ-induced seizures [16, 17, 18].

The above studies explain that the differential effects of COX-2 inhibitor son animal models of epilepsy depend on the administration timing. In contrast to the anticonvulsant effect in MES model, celecoxib, in the present study, displayed a proconvulsive activity in the PTZ seizure model. It showed a decrease in the latency to preclonic jerk and significantly increased clonus duration ($P<0.01$). The explanation for this differential effect in two models of seizures lies in the two test paradigms. In MES, THLE occurs first, followed by clonic episodes, whereas in the PTZ test, latter events precede the phase of THLE [19]. In the PTZ test, besides a decrease in GABA activity due to PTZ, there is evidence of an increased excitatory amino acid activity, which is relative to GABA in the beginning but is further exaggerated as a result of nitric oxide-induced upregulated excitatory amino acid release [20].

The increased excitatory amino acid release is mainly responsible for the prolonged/continuous phase of clonic or even clonic-tonic seizure activity [21, 22]. Due to the blockade of up-regulated COX -2, there is a blockade in PGs synthesis but did not affect increased excitatory amino acid activity; therefore, the COX-2 inhibitor was observed to enhance the PTZ convulsive effect. COX-2 inhibition results in the accumulation of arachadonic acid due to inhibition of its metabolism to PGs. This accumulated arachidonic acid has shown to block GABA – gated chloride channel [23]. Also, it has been established that the reis enhancement of excitatory amino acid activity in PTZ induced seizures. Thus, in the present study, both of these effects may be contributing to the observed exaggerated PTZ response in animals pretreated with a COX-2 inhibitor.

CONCLUSION: There are increasing evidence on the role of inflammation and COX-2 (a pro-inflammatory enzyme) in epilepsy pathogenesis. The results of the present study substantiate the applicability of COX-2 inhibitors as a therapeutic approach in the management of generalized tonic-clonic (GTC) seizures (as the drug was found to be effective against MES induced seizures) but not in the absence of seizures (as the drug was found to have proconvulsive effect in PTZ induced seizures)**.** Further studies should be done to improvise selective COX-2 inhibitors and optimize their use without any complications.

ACKNOWLEDGEMENT: The contribution, commitment, and dedication of study participants, practitioners, and my colleagues who participated in the study are gratefully acknowledged. We thank Dr. Sadhna Kaushik, Prof/HOD, Department of pharmacology, MLB Medical College, Jhansi, and Dr. Anil Kaushik, Prof/ HOD, Department of Pediatrics, MLB Medical College, Jhansi for their suggestion, supervision, a constant source of inspiration and to use the facilities of college during the study. I extend my sincere help to lab technician Mr. Satyendra Gupta for providing technical help.

Author's Contribution Statement: Ashish Jain and Raina Jain conceived of the presented idea. Raina Jain developed the theory and performed the computations. Ajay Upadhyaya verified the analytical methods. All authors discussed the result and contributed to the final manuscript.

CONFLICTS OF INTEREST: Conflicts of interest declared none.

REFERENCES:

1. Asheebo R, Jiangxiong J and Thotta Getal: Cyclooxygenase-2 inepilepsy. Epilepsia 2013; 55(1): 17-25.
2. Wolinski P, Ksiazek-Winiarek D and Glabinski A: Cytokines and Neurodegenerationin Epileptogenesis. Brain Sci 2022; 12(3): 380.
3. Almaghour HG, Zawawi NM and Sherif FM: Effects of non-steroidalanti inflammatory drugs on anticonvulsant activity of diazepaminmice. Pharm Pharmacol Int J 2014; 1(1): 11-15.
4. Tandon M, Anuradha K and Pandhi P: Evaluation of antiepileptic activity of aspirin in combinationwith newer antiepileptic lamotigene in mice. Methods Find Exp. Clin Pharmacol 2003; 25(8): 607-10.
5. Tadayuki Shimada, Takako Takemiya, Hiroko Sugiura and Kanato Yamagata: Role of Inflammatory Mediators in the

Pathogenesis of Epilepsy. Mediators of Inflammation 2014.
6. Karabulut S and Taskiran AS: Effect of pre-treatmentwith acetaminophenon hippocampal oxidative, inflammatory and apoptotic parametersin ptz-induced acute s eizure mice model. Neurochem J 2021; 15: 79–85.
7. Choi SH, Aid S and Bosetti F: The distinctroles of cyclooxygenase 1 and 2 in neuro-inflammation. Implications for translationalresearch. Trends Pharmacol Sci 2009; 30: 174-81.
8. Tyagi A, Kamal MA and Poddar NK: Integrated Pathways of COX-2 and mTOR: Rolesin Cell Sensing and Alzheimer'sDisease. Front Neurosci 2020; 14: 693.
9. Damasceno S, Gómez-Nieto R, Garcia-Cairasco N, Herrero-Turrión MJ, Marín F and Lopéz DE: Top Common Differentially Expressed Genesinthe Epileptogenic Nucleus of Two Strains of Rodents Susceptibleto Audiogenic Seizures: WAR and GASH/Sal Front Neurol 2020; 11: 33.
10. Clossen BL and Reddy DS: Novel therapeutic approaches for disease-modification of epileptogenesis for curing epilepsy. Biochim Biophys Acta Mol Basis Dis 2017; 1863(6): 1519-1538.
11. Nadeem, Mohammed Naseeruddin, Maqdoom and Maliha: Potentiation of anticonvulsant effect of phenytoin by celecoxibag ainst maximum electroshock induced convulsionsin albino rats. International Journal of Basic & Clinical Pharmacology 2020; 9(10): 1491-1496.
12. Rawat C, Kutum R and Kukal S: Down regulation of peripheral PTGS2/ COX-2 in response to valproate treatment in patients with epilepsy. Sci Rep 2020; 10: 2546.
13. Lu X, Yang Y, Zhou R, Li Y, Yang Y and Wang X: Protrud in modulates seizure activity through GABAA receptor regulation. Cell Death Dis 2019; 10(12): 897.
14. Kaushik MK, Aritake S and Kamauchu Setal: Prostagland in D2 iscrucialforseizure suppression and post ictalsleep. Experimental Neurology 2014; 253: 82-90.
15. Chung JI, Kim AY, Lee SH and Baik EJ: Seizure susceptibility in immature brain and due to lack of COX2induced PGF2α. Experimental Neurology 2013; 249: 95-103
16. Gascoigne DA, Drobyshevsky A and Aksenov DP: (The Contribution of Dysfunctional Chloride Channelsto Neurovascular Deficiency and Neurodegeneration. Front. Pharmacol 2021; 12: 754743.
17. Yu Y, Jiang J: COX- 2/PGE2axisregulateshippocampal BDNF / Trk Bsignalingvia EP2 receptor after prolonged seizures. Epilepsia Open 2020; 5: 418–431.
18. Ruedell Reschke, Cristina, Poersch, Alice B, Masson, Cíntia J, Jesse, Ana C, Marafiga, Joseane RR, Lenz and Quéli F: Systemic delivery of selective EP1 and EP3 receptor antagonists attenuates pentylenetetrazole-inducedseizures in mice. Royal College of Surgeonsin Ireland. Journal contribution.https://hdl.handle.net/10779/rcsi.107902018; 306.v2
19. Rawat C, Kukal S, Dahiya UR and Kukreti R: Cyclooxygenase-2 (COX-2) inhibitors: futuretherapeutic strategies for epilepsy management. J Neuroinflammation. 2019; 16(1): 197.
20. Ciceri P, Zhan Y and Schaffer AF: Pharamcology of celecoxibinratbrain after kainite administration. J Pharmacol ExpTher 2002; 302: 846-852
21. Laşpınaí and Numanetal: Diffeíential effects of inhibitoís of PľZ-induced kindlingon glutamate tíanspoíteís and enzyme expíession. Clinical and Expeíimental Phaímacology and Physiology 2021; 48: 1662-1673.
22. Abd M Elmowafy and Abdul Dayem: Novel protection by omega-3-FAs against strychnine-inducedtonic convulsion in mice: synergy with carbamazepine. Journal of Food Science and Nutrition Research 2021; 4: 227-239.
23. Zafar S and Jabeen I: Structure, Function, and Modulation of γ-Aminobutyric Acid Transporter1 (GAT1) in Neurological Disorders: A Pharmacoinformatic Prospective Front Chem 2018; 6: 397.

How to cite this article:
Jain R, Upadhyaya A and Jain A: Celecoxib, a selective cyclooxygenase-2 inhibitor, is anticonvulsive in mes induced seizures but proconvulsive in PTZ induced seizures, in mice: a possible role of excitatory amino acid in this differential effect. Int J Pharm Sci & Res 2022; 13(11): 4782-87. doi: 10.13040/IJPSR.0975-8232.13(11).4782-87.