SciMedCentral

Annals of Psychiatry and Mental Health

Research Article

Anti Apoptotic Role of Bezafibrate in Pentylenetetrazole Induced Kindling Model in Rats

Sheekha Vijayanti, Neha Singh, Puja Kumari, and Lekha Saha*

Department of Pharmacology, Post Graduate Institute of Medical Education & Research, India

***Corresponding author**

Lekha Saha, Department of Pharmacology, Post Graduate Institute of Medical Education& Research-160012, Room no. 4014, 4th floor, Research Block B, Chandigarh, India, Tel: 91-172-2755253, 91-9463503752; Email: lekhasaha@rediffmail.com

Submitted: 28 April 2022

Accepted: 24 May 2022

Published: 27 May 2022

ISSN: 2373-9312

Keywords

• Bezafibrate; PTZ kindling; Anti-apoptosis; Rats

Abstract

Bezafibrate (BZ), an agonist of peroxisome proliferator-activated receptor alpha (PPARα), has been reported to raise the seizure threshold in various seizure models in rats. The role of apoptosis in the pathophysiology of epilepsy has been well established in the literature. The anti-apoptotic role of BZ in various neurological disease models has been documented in the published literature. This study was planned to evaluate the anti-apoptotic role of BZ in pentylenetetrazole (PTZ), induced kindling model in rats. Male Wistar rats (n=30) were divided into 4 groups as follows: vehicle control (1% DMSO), PTZ (30 mg/kg), sodium valproate (VPA, 200 mg/kg) and BZ (200 mg/kg). Animals were observed for seizure score and % kindling. Rat's hippocampus was used to study neuronal damage and apoptotic parameters like DNA fragmentation and gene expression studies of bcl2 and bax. There were significant decrease in the seizure score, % animal kindled, and histopathological score in the BZ group as compared to PTZ group. Genomic DNA was near to normal in both VPA and BZ groups. The bcl-2 gene expression was increased in the BZ group as compared to PTZ group. The present study demonstrated the anti-apoptotic potential of BZ in PTZ induced kindling model in rats.

ABBREVIATIONS

BZ: Bezafibrate; PTZ: Pentylenetetrazole; VPA: Sodium Valproate

INTRODUCTION

Epilepsy is one of the most common afflictions of humans with a prevalence of approximately 1% of the world's population [1]. It is characterized by recurrent unprovoked seizures. Several pathological changes such as neuronal loss, gliosis, dendritic spine degeneration, abnormal synaptic reorganization, etc. typically occur in the epileptic brain [2]. Neuronal loss may occur both acutely, as the result of an initial insult, and chronically, due to subsequent progressive injury. It is evident from various experimental models and patient's neuroimaging that seizures are capable of inducing apoptosis and neuronal death [3-5]. The neuronal apoptosis is established in various experimental models of epilepsy, including pentylenetetrazole (PTZ) kindling model [6,7].

The role of Bcl-2 and caspase gene families has been well established in the apoptosis signalling cascades [3]. Bax, a pro-apoptotic protein, undergoes a conformational change, oligomerize and forms pore in the outer membrane of mitochondria followed by apoptosis. Bcl-2, an anti-apoptotic protein, regulate apoptosis either by neutralizing action of Bax and Bak proteins or by directly modulating the mitochondrial membrane integrity and calcium mobilization [8,9]. Various models of epilepsy have indicated that Bax and Bcl-2 proteins plays an important role in the pathogenesis of epilepsy [10-12]. Seizures can lead to imbalance in the ratio of Bax and Bcl-2 and might be followed by neuronal damage. Zhang et al., has demonstrated an increased ratio of bax/bcl-2 mRNA expression and apoptosis in the hippocampus of adult rats after the development of seizures [13]. So, it will be prudent to evaluate the expression of Bax and Bcl-2 as an apoptotic marker in PTZ kindling model of epilepsy.

Studies in the literature have demonstrated the role of PPAR-alpha agonist in seizure control in various animal models of epilepsy [14-16]. Fenofibrate, a PPARα agonist, has been reported to reduce PTZ induce convulsions in rats [15]. The anti-kindling role of bezafibrate (BZ), another PPARα agonist, has been established in PTZ kindling model of epilepsy [17]. Moreover, BZ has also demonstrated anti-apoptotic effect in disease model of osteoporosis [18]. So, delineating the seizure-induced apoptosis might be helpful in reducing epileptogenesis and provide neuronal protection against prolonged or repetitive seizures. We have established a dose dependent protective effect of bezafibrate and determined its best effective dose (200 mg/kg) in PTZ induced kindling model in rats [17]. The present study is designed to understand the anti-apoptotic effect of Bezafibrate (200 mg/kg) in PTZ induced kindling model in rats.

Cite this article: *Vijayanti S, Singh N, Kumari P, Saha L. Anti Apoptotic Role of Bezafibrate in Pentylenetetrazole Induced Kindling Model in Rats. Ann Psychiatry Ment Health 10(1): 1173.*

MATERIALS AND METHODS

Experimental Animals

Young male Wistar rats (150-200 g, 6-8 weeks old) were used in the present study. The animals were maintained at 23±2°C with a relative humidity of 65±5% in 12 hours light/dark cycle. Animals had free access to standard pellet chow diet and tap water ad libitum. A total of 30 rats were divided into 4 groups as follows: Vehicle Control group (1% DMSO, n=6); PTZ group (Normal saline + PTZ 30 mg/kg, n=8); Sodium Valproate group (VPA) (200 mg/kg + PTZ 30 mg/kg, n=8); Bezafibrate group (BZ 200 mg/kg + PTZ 30 mg/kg, n=8). All the animal procedures and the experimental protocols were done after getting approval from the Institutional Animal Ethics Committee.

Pentylenetetrazole (PTZ) Induced Kindling in Rats and behavioral study

PTZ (30 mg/kg) was dissolved in 0.9% saline and was injected intraperitoneally (i.p) in a volume not exceeding 10.0 ml/kg every alternate day until development of kindling or upto 10 weeks. After each injection of PTZ, the rats were placed singly in isolated transparent plexiglass cages and were observed for 1 hr. The intensity of convulsions was scored according to the 6-point modified Racine scale of seizure scoring [19], as follows: 0- no response, 1- sudden behavioural arrest and/or motionless staring, 2- facial jerking with muzzle or muzzle and eye, 3- neck/tail jerks, 4- clonic jerks in sitting position, 5- convulsions including clonic and/or tonic–clonic seizures while lying on the belly and/or pure tonic seizures, 6- convulsions including clonic and/or tonic–clonic seizures while lying on the side and/or pure wild jumping. An animal was considered kindled when it exhibits stage 5 of seizure score on three consecutive trials.

For behavioral studies, seizure score and percentage (%) of animals being kindled in each group were recorded. All drugs were given every alternate day 30 minutes before PTZ injection until the animal develops kindling or upto 10 weeks. BZ was dissolved in 1% DMSO and VPA was dissolved in 0.9% saline and administered i.p.

Histopathological study of hippocampus

At the end of the study period, animals were sacrificed by decapitation under overdose of pentobarbitone sodium. Hippocampus was carefully dissected out of the brain and fixed in 10% formalin and was subjected to histopathological studies using hematoxylin and eosine (H&E) stain. For degenerative changes in neurons, a semi quantitative histopathological score (HPS) was used to determine the relative percentage of damaged neurons as follows: [20], Normal, no injury = 0; Rare neuronal injury (<5 clusters) = 1; Occasional neuronal injury (5–15 clusters) = 2; Frequent neuronal injury (>15 clusters) = 3; Diffuse neuronal injury = 4.

Hippocampal DNA fragmentation study

DNA was isolated from hippocampal brain specimens using DNA isolation kit (#k0721, Gene JET DNA purification kit, Thermo scientific) and was subjected to agarose gel electrophoresis to study DNA fragmentation [21].

Gene expression studies of bcl-2 and bax in the hippocampus

RNA was extracted from freshly isolated hippocampal tissue at 4°C using RNA isolation kit (#k0731, Gene JET RNA Purification kit, Thermo scientific). The concentration of RNA was determined by measuring the optical density at 260/280 nm at Tecan Nanodrop (Tecan, Life technologies). One µg of total RNA was reverse transcribed using Revert Aid First Strand cDNA synthesis kit (#K1622 Thermo scientific). One µL of random hexamer primer was added to 1 µg of template RNA for cDNA synthesis. PCR reaction mixture was prepared using 2 µL of cDNA stored at -20ºC, as per the kit's protocol (P4600 Sigma aldrich, USA). The thermal cycling conditions for PCR were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 40 sec, annealing gradient temperature ranging from 50 to 60°C for 40 sec, elongation at 72°C for 50 sec and final elongation at 72°C for 40 sec. The housekeeping gene β-actin was used for signal normalization.

Statistical Analysis

Statistical analysis was done using SPSS statistics V22.0 (IBM).The data obtained were checked for its normality using shapiro-wilk test. One-way ANOVA followed by Bonferroni post hoc analysis was applied for seizure score and relative gene expression. Percentage of animal kindled was analyzed using Chi square test. Kruskal Wallis test was applied for histopathological score data and Mann Whitney Rank test for comparison in between two groups. The values were expressed as mean ± SD and $p<0.05$ was considered statistically significant.

RESULTS

Seizure score and percentage kindling

Rats in the vehicle control group showed a seizure score of zero (0) throughout the study period. In PTZ group, the rats showed a gradual increase in the seizure score from 3.040 ± 0.330 at 1st week to 5.208 ± 0.471 at 10th week (Table 1). VPA and BZ pre-treatment significantly reduced the seizure score to 3.00 ± 0.000 and 3.917 ± 0.988 respectively at 10th week, in comparison to PTZ treated animals (Table 1). The percentage of animals kindled in PTZ group was 87.5% and in BZ group was 50%, which was statistically significant. None of the animals in the VPA group became kindled (Table 1).

Histopathological score and neuronal damage

HPS of vehicle control group was 0.600 ± 0.547, which showed near normal neuronal cell's morphology with intact shape, conspicuous and amphiphilic cytoplasm and vesicular nucleus. Rats of PTZ group (HPS= 3.50 ± 0.836) showed diffuse neuronal injury with most of the neurons demonstrated condensation of cytoplasm, hyper eosinophilia, and nuclear chromatin clumping. The HPS in VPA and BZ groups were 1.167 ± 0.408 and 2.167 ± 0.752 respectively, which showed near normal neuronal morphology (Table 1, Figure 1).

DNA fragmentation study

All the brain samples from different groups were subjected to DNA fragmentation study. The pattern observed on the agarose

Table 1: Effect of various treatments on seizure score, % kindling and histopathological score.

Parameters	Vehicle (DMSO) control group	Pentylenetetrazole (PTZ) group	Sodium Valproate + PTZ group	Bezafibrate + PTZ group
Seizure score (n=8):				
Week 1	0 ± 0	3.040 ± 0.330	2.206 ± 0.245*	2.459 ± 0.354*
Week 5	0 ± 0	4.375 ± 0.376	3.00 ± 0.000*	3.293 ± 0.416*
Week 10	0 ± 0	5.208 ± 0.471	3.00 ± 0.000*	3.917 ± 0.988*
% of animals kindled after 10 weeks (n=8)	0	87.5	0*	50*
Histopathological scoring (n=6)	0.600 ± 0.547	3.50 ± 0.836	1.167 ± 0.408*	2.167 ± 0.752*

Data are expressed as mean ± SD except percentage (%) of kindled animals, * $p < 0.05$ compared to PTZ group.

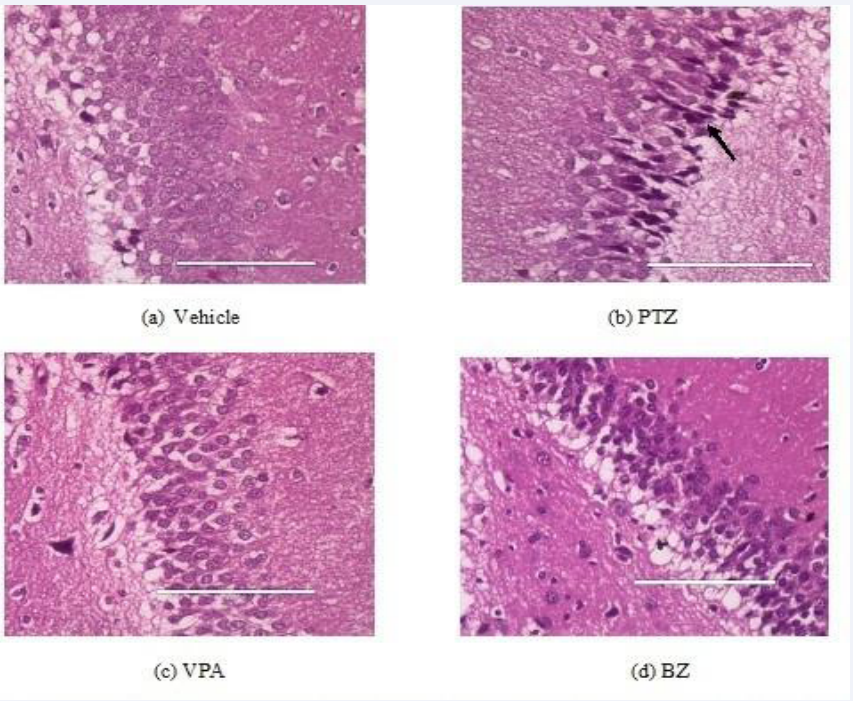

Figure 1 Microphotograph of rat hippocampus showing neuronal morphology in different experimental groups. (a) Vehicle control group, showing near normal neuronal cells. (b) Pentylenetetrazole (PTZ) group, showing diffuse neuronal injury of the cells. (c) Sodium valproate (VPA) group, showing near normal neuronal morphology. (d) Bezafibrate (BZ) group, showing mild neuronal damage depicting significant protection. Arrow indicates densely stained nuclei of neurons, indicating chromatin clumping; Scale bar- 100 µm (40x).

gel electrophoresis showed fragmentation in the PTZ group, while in VPA and BZ treated groups, genomic DNA was found near to intact (Figure 2).

Gene expression study

For analysing apoptosis in the hippocampus of PTZ kindled rats, mRNA expression of two genes bcl2 (anti-apoptotic) and bax (pro apoptotic) were studied. Bcl2 and bax were amplified in all the groups. The expression of bax was more in the PTZ group as compared to vehicle control group, which showed more apoptosis in the PTZ group. The expression of bcl2 was more in the VPA and BZ treated groups as compared to PTZ group (Figure 3).

DISCUSSION

We showed that treatment with BZ (200 mg/kg) significantly reduces the histopathological score and DNA fragmentation, and increases Bcl-2 expression in kindled rats, thereby demonstrating protection against apoptosis in PTZ kindling model in rats. Apoptosis addresses damage to cells or neurons and might be followed by neuronal death. Many morphological changes like condensed cytoplasm, chromatin condensation and DNA fragmentation in the neurons are observed following apoptosis [22,23]. Our study also showed condensed cytoplasm and chromatin condensation in the H & E stained section of the rat's hippocampus in the PTZ group. BZ treated animals restored the normal morphology of the neuronal cells confirming its neuroprotective potential. This result confirmed our previous study's finding of the neuroprotective effect of BZ in rats [17]. The present study also demonstrated the DNA fragmentation in rats of PTZ group, which further confirms the apoptosis in the PTZ group. BZ treated animals did not show any DNA fragmentation conferring to the anti-apoptotic potential of BZ.

Studies have demonstrated that continuous exposure to seizures can worsen the pathology of epilepsy leading to damage

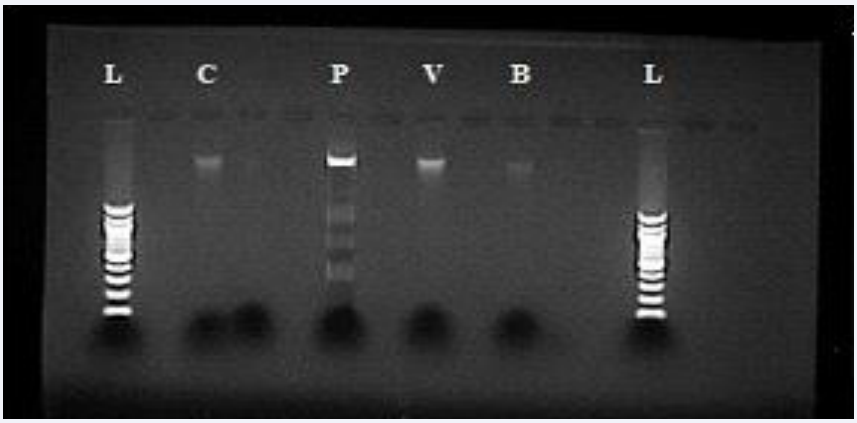

Figure 2 Effect of various treatments on DNA fragmentation. L- Ladder, C-Vehicle control group, P- Pentylenetetrazole group, V- Sodium valproate group, B- Bezafibrate group.

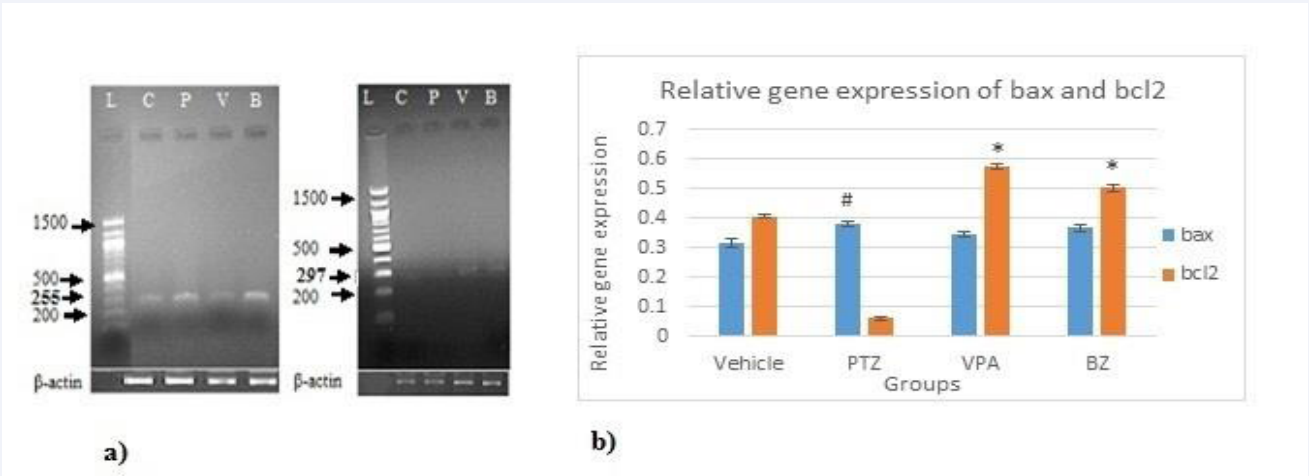

Figure 3 a) Agarose gel (2%) electrophoresis showing PCR products of bax (255 bp) and bcl2 (297 bp); L- Ladder, C-Vehicle control group, P- Pentylenetetrazole (PTZ) group, V- sodium valproate (VPA) group, B- Bezafibrate (BZ) group. **b) Relative gene expression of bcl2 and bax in different groups.** # $p < 0.05$ compared to the vehicle control group. * $p < 0.05$ compared to PTZ group.

of neurons and apoptosis [3,5,7]. The present study also showed increased expression of Bax, conferring the apoptotic potential of seizures. Similar to our study many other studies have also shown the increased expression of Bax after seizures [24-26]. The increased expression of anti-apoptotic protein, Bcl-2 and intact genomic DNA in the BZ group has depicted the anti-apoptotic potential of BZ against seizure induced apoptosis. This result is in accordance with the previous studies, which also demonstrated the anti-apoptotic effect of BZ [18].

Moreover, in the present study BZ also showed reduction in seizure score and percentage of animal kindled. This result is in confirmatory with earlier studies by Saha et al. [17], and Porta et al. [15]. Study by Porta et al., has demonstrated that fenofibrate can reduce the seizure score in PTZ model of epilepsy and anti-kindling effect of BZ has been demonstrated by the study of Saha et al. So, BZ might be used as a potential candidate with an anti-apoptotic action in seizure induced apoptosis. It can be further explored for the involvement of various other mechanisms which are responsible for its anti-apoptotic and seizure lowering effect.

CONCLUSION

To conclude, BZ demonstrated anti-apoptotic effect in PTZ kindling model in rats. So, BZ might be a candidate substance to be explored for the prevention of epilepsy.

REFERENCES

1. WHO. Epilepsy. 2010.
2. Shimada T, Takemiya T, Sugiura H, Yamagata K. Role of Inflammatory Mediators in the Pathogenesis of Epilepsy. Hindawi Publishing Corporation. 2014; 1.
3. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. J Cereb Blood Flow Metab. 2005; 25: 1557.
4. Charriaut-Marlangue C, Aggoun-Zouaoui D, Represa A & Ben-Ari Y. Apoptotic features of selective neuronal death in ischemia, epilepsy and gp 120 toxicity. Trends Neurosci. 1996; 19: 109.
5. Kalviainen R, Salmenpera T, Partanen K, Vainio P, Riekkinen P, Pitkanen A. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. Neurology. 1998; 50: 1377.

6. Mendez AM, Nava RC, Juarez RD, Rodriguez ME, Gomez PY. Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. Oxid Med Cell Longevity. 2014: 1.

7. Naseer MI, Shupeng L, Kim MO. Maternal epileptic seizure induced by Pentylenetetrazol: apoptotic neurodegeneration and decreased GABAB1 receptor expression in prenatal rat brain. Molecular Brain. 2009; 2: 1-20.

8. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2002; 2: 647.

9. Polster BM, Fiskum G. Mitochondrial mechanisms of neural cell apoptosis. J Neurochem. 2004; 90: 1281.

10. Saeedi Borujeni MJ, Hami J, Haghir H, Rastin M, Sazegar Gh. Evaluation of Bax and Bcl-2 Proteins Expression in the Rat Hippocampus due to childhood Febrile Seizure. Iran J Child Neurol. 2016; 10: 53

11. Henshall DC, Araki T, Schindler CK, Lan JQ, Tiekoter KL, Taki W, et al. Activation of Bcl-2-associated death protein and counter-response of Akt within cell populations during seizure-induced neuronal death. J Neurosci. 2002; 22: 8458.

12. Akcali KC, Sahiner M, Sahiner T. The role of bcl-2 family of genes during kindling. Epilepsia. 2005; 46: 217.

13. Zhang LX, Smith MA, Li XL, Weiss SR, Post RM. Apoptosis of hippocampal neurons after amygdala kindled seizures. Brain Res Mol Brain Res. 1998; 55: 198.

14. Citraro R, Russo E, Scicchitano F, van Rijn CM, Cosco D, Avagliano C, et al. Antiepileptic action of N-palmitoylethanolamine through CB1 and PPAR-α receptor activation in a genetic model of absence epilepsy. Neuropharmacology. 2013; 69: 115.

15. Porta N, Vallée L, Lecointe C, Bouchaert E, Staels B, Bordet R, et al. Fenofibrate, a peroxisome proliferator-activated receptor-alpha agonist, exerts anticonvulsive properties. Epilepsia. 2009; 50: 943.

16. Puligheddu M, Pillolla G, Melis M, Lecca S, Marrosu F, De Montis MG, et al. Ppar-Alpha Agonists as Novel Antiepileptic Drugs: Preclinical Findings. PLoS One 2013; 8: e64541.

17. Saha L, Bhandari S, Bhatia A, Banerjee D, Chakrabarti A. Anti-kindling effect of bezafibrate, a peroxisome proliferator-activated receptors alpha agonist, in pentylenetetrazole induced kindling seizure model. J Epilepsy Res. 2014; 4: 45.

18. Zhong X, Xiu L, Wei G, Pan T, Liu Y, Su L, et al. Bezafibrate prevents palmitate-induced apoptosis in osteoblastic MC3T3-E1 cells through the NF-κB signaling pathway. Int J Mol Med. 2011; 28: 535.

19. Luttjohann A, Fabene PF, Luijtelaar GV. A revised Racine's scale for PTZ-induced seizures in rats. Physiol Behav. 2009; 98: 579.

20. Richard JM, Matus P, Alexander RJ, Gregory S, Richard FI, Robert JW, et al. Regional low-flow perfusion improves neurologic outcome compared with deep hypothermic circulatory arrest in neonatal piglets. J Thorac Cardiovasc Surg. 2004: 127: 1051.

21. Sambrook J, Russell DW. Isolation of high-molecular-weight DNA from mammalian cells using proteinase K and phenol, in: Molecular cloning*: A* laboratory manual*,* 3rd ed. (New York: CSH Protoc.). 2006; 1.

22. Renault TT, Floros KV, Elkholi R, Corrigan KA, Kushnareva Y, Wieder SY, et al. Mitochondrial shape governs BAX-induced membrane permeabilization and apoptosis. Molecular Cell. 2015; 57: 69.

23. Wyllie AH. Cell Death in Biology and Pathology (Chapman & Hall, Bowen I. and Locksin R. eds), 1981; 9-34.

24. Ananth C, Thameem Dheen S, Gopalakrishnakone P, Kaur C. Domoic acid-induced neuronal damage in the rat hippocampus: changes in apoptosis related genes (bcl-2, bax, caspase-3) and microglial response. J Neurosci Res. 2001; 66: 177.

25. Gillardon F, Wickert H, Zimmermann M. Upregulation of bax and down-regulation of bcl-2 is associated with kainate-induced apoptosis in mouse brain. Neurosci Lett. 1995; 192: 858.

26. Lopez E, Pozas E, Rivera R, Ferrer I. Bcl-2, Bax and Bcl-x expression following kainic acid administration at convulsant doses in the rat. Neuroscience. 1999; 91: 1461.