Advances in Bioresearch
Adv. Biores., Vol 15 (4) July 2024:111-118

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.4.111118

Advances in Bioresearch

ORIGINAL ARTICLE

Screening of Anti-Epileptic Activity of Brucine Using PTZ Induced Adult Zebrafish Model

Kenz Mudalvan M[1], Narayanan J[1*] And Chitra V[1]
[1]Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur 603203, Tamilnadu, India.
***Corresponding author's** e-mail address: narayanj@srmist.edu.in

ABSTRACT
About 50 million individuals with epilepsy globally, 80% live in poor nations, according to the World Health Organization (WHO). Epilepsy is multi-factorial chronic neurological disorder. The pathophysiology of epilepsy is not fully explored. According to the research evidence it is due to hyper excitability and hypersynchronous electrical discharge in brain neurons, leading to abnormal autonomic, motor, and sensory disturbances and it is induced by removing extracellular magnesium, increasing potassium concentration, inhibiting sodium pump, or antagonizing GABA receptors in neurons. Genetic pathologies cause epilepsy in various ways. The genetic basis of epilepsy syndromes, with both monogenic and polygenic mutations contributing to epilepsy. Multiple investigations indicate that the hippocampal neurons, which are essential for the loop's recovery after repeated convulsions, may be harmed by epileptic seizures. JNK pharmacological inhibition is a well-established technique for avoiding of neuronal death. Brucine an alkaloid found in plants, has been shown to have cytotoxic, antiproliferative, antitumor, and antiangiogenic effects. Animals (n = 6/group) were given with 10µl and 20µl of test drug and 10µl of standard drug for 14 days. On 15th day fishes are treated PTZ solution is given to induce convulsion. Results shows the convulsion latency of the fish is reduced when compared with negative control and standard group. On comparing the low and high dose of brucine with standard drug phenytoin it shows that the three phases of convulsion (S1, S2, S3) time is reduced in low and high dose of brucine when compared with phenytoin.
KEY WORDS: *Zebra fish, Anti-convulsant, Pentylene-tetrazole, JNK, Auto dock*

Received 30.03.2024 Revised 23.04.2024 Accepted 24.06.2024

How to cite this article:
Kenz Mudalvan M, Narayanan J And Chitra V. Screening of Anti-Epileptic Activity of Brucine Using PTZ Induced Adult Zebrafish Model. Adv. Biores. Vol 15 [4] July 2024. 111-118

INTRODUCTION

According to the International League Against Epilepsy (ILAE; 1993), epilepsy is a disorder marked by repeated seizures that are not immediately caused by anything. Of the 50 million individuals with epilepsy globally, 80% live in poor nations, according to the World Health Organization (WHO). By accounting for 7,307,975 disability adjusted life years (DALYs) in 2005, epilepsy was projected to represent 0.5% of the worldwide illness burden. Epilepsy is multi factorial chronic neurological disorder. Pathophysiology involved in epilepsy is hyper excitability and hypersynchronous of electrical discharge in the neurons in the brain which alters the concentration of sodium, potassium and calcium ions in the cell and it also releases neurotransmitter in the neuron which leads to cause brain disfunction which interrupt normal function and it is episodes of abnormal autonomic, motor, and sensory disturbances in the brain. [1] .The epileptic activity that recorded in the cortical and hippocampal neuron of brain is interictal spike which is a rhythmic burst of action potential caused by the repetitive excitation.

The main cause for seizure generation is the synchronized and rhythmic interaction between the excitatory and inhibitory neurons and membrane conductance. when there is a loss of balance between the excitatory and inhibitory neuron seizures are produced. Seizures are two types one is partial (simple or complex) another one is generalized. Generalised further categories including absence, tonic, clonic, tonic-clonic, myoclonic, and febrile seizures. The main cause of Epileptiform is decreasing in extracellular magnesium, increasing extracellular potassium concentration and inhibition of the sodium pump or antagonizing GABA receptors in the neurons.[2]

Seizure can cause temporary loss of consciousness or abnormal motor activity and also cause minor involuntary movement to whole body convulsion. Anti-epileptic drug mainly acts on voltage-dependent sodium, potassium and calcium channels, γ-aminobutyric acid type A (GABA-A) receptors, GABA-metabolizing enzymes and GABA transporters, excitatory amino acid receptors or synaptic proteins. [3]
Action potential in the brain is initiated and propagated by voltage dependent Na+ channels making them key factor for neuronal excitability and initially axon and soma depolarized for a longer period of time which thereby stimulate continuous burst of action potential. The burst generated from the dendrite to the soma can back propagate into dendrites which creates dendritic spikes [4].
Genetic pathologies cause epilepsy in Nemours ways which includes abnormal g-aminobutyric acid [GABA] receptor subunits occurs in Angelman syndrome to abnormal mutation of ionic channel (e.g., potassium channel mutations) is known as benign familial neonatal epilepsy (BFNE). Similarly, acquired cerebral injuries can alter the brain function (e.g., structural modification of hippocampal structure followed by prolonged febrile seizures or head trauma).[5] Seizures are more prevalent in newly developing brain due to various physiological reasons. the reason for higher excitation and seizure formation in developing brain is due to development excitatory synaptic function occurs first then inhibitory synaptic function develops. So, in early stages the neurotransmitter GABA causes excitation instead of inhibition. [6]
The above information's helps us to understand why seizures are common in developing brain and it also cause less structural damage in newly forming brain than in the adult brain. In addition, both monogenic and polygenic mutations in brain can cause epilepsy.[7]
MacDonald and Barker stated the acute effect of PTZ on cultured mammalian neuron. the electro-physiological investigation of PTZ reveals that the pharmacological effect of PTZ is blockage of benzodiazepine recognition sites in GABA receptor, which causes inhibition of transmission mediated by γ-aminobutyric acid (GABA). The duration of the PTZ-treatment regulates the activation of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding [8]. One of the studies in PTZ-kindled model reveals the metabolic disturbances in the temporal lobe in the.[9] Hyperactive MAPK affect RBPs and RBP-mediated expression control, influencing transcript transport, stability, and translation. This spatially restricted mis regulation may accelerate neuronal firing, leading to seizures. [10]
JNK signalling pathway it causes the neuronal cell death by activating intrinsic and extrinsic pathway. Since it stimulates apoptotic signalling by upregulating proapoptotic genes JNK play a key role in epilepsy. We believe that JNK pathway inhibitors might be a novel class of adjuvant medications that reduce neuronal death caused by seizures and could be a therapeutic approach for epilepsy therapy. JNK pharmacological inhibition is a well-established technique for avoiding of neuronal death. JNK can be inhibited through number of ways, a JNK inhibitor that selectively inhibits JNK activity to cease c-Jun phosphorylation. The inhibitor that competes with MLK kinases for the ATP binding site, hence activating the JNK pathway.[11]
Brucine is an alkaloid found in plants, isolated from dried Strychnos nux-vomica L. seeds, which belongs to (Loganiaceae). In the Chinese medicine used for reliving pain and promotes blood circulation and it also has biological activities.[12] Preliminary studies suggest cytotoxic, antiproliferative, antitumor, and antiangiogenic effects of brucine. [13]
In silico method plays a vital role in the drug discovery and drug development. In *in silico* method using molecular docking technology various ligand-protein adducts were generated and based on the binding energy obtained docking program the promising compound were taken for further studies. AUTODOCK 4.2 and AUTODOCK TOOLS is a software used to predict the correct ligand-protein binding energy.[14] Binding interaction between the ligand and the protein is obtained using BIOVIA DISCOVERY STUDIOS. So estrogenic receptor, caspase 3 receptor, human mitochondrial aldehyde dehydrogenase., HDAC, Ach E were taken as the target for *Insilico* studies. In this study, we are going to evaluate the anti-convulsant activity of the drug brucine in zebra fish by comparing the drug treated groups with the standard group.
The zebrafish (*Danio rerio*). Zebra fish shows numerous genetic and structural similarities with mice and humans. It possess higher vertebrates like higher macromolecule permeability and tight junction-based blood–brain barrier, which makes this promising a model for AED discovery and high throughput screening applications. [15]
The drug is dissolved in water through ultrasonication and given to the adult zebrafish through oral route for 14 days. On 15th day using PTZ seizure is induced through oral route and the stages of the seizure is recorded and zebrafish brain is dissected and taken for histopathological examination.

MATERIAL AND METHODS

IN-SILICO DOCKING SCREENING

The compound of interest that taken for *in-silico* screening is brucine. The ligand is made to bind with the protein to assess the molecular binding score. The ADMET property, molecular property and the bioactivity score of the ligand were obtained.[14]

DOCKING ANALYSIS

The proteins which are involved in the pathophysiology of epilepsy was downloaded using RCSB PDB database in PDB format. The binding energy and interaction between ligand and protein were determined using Auto dock 4.1.

LIGAND PREPARATION

The ligand's molecular structure was developed using chem sketch software and saved in mol format. Virtual screening tool is used for energy minimization and optimization by upload the generated structure to open babel and the structure is converted to (.pdbqt) format. For successful running of Auto dock 4.1 program, it requires (.pdbqt) format. [16]

PROTEIN PREPARATION

Proteins are the macromolecules which plays a pivotal role in the pathophysiology of the diseases which acts as the target for the drug. The x-ray crystallography 3D structure of the protein is download from the RCSB PDB database. The water molecules, hetero atoms and other residues are removed and visualized using the Biovia Discovery Studios 2021. Polar hydrogens were added and the derived protein is saved in pdb format which is upload to auto dock 4.2 and converted to pdbqt format.[11][17][2]

VIRTUAL SCREENING OF DOCKING ANALYSIS

Virtual screening is a mostly used tool to identification novel compound. Virtual screening is divided into two categories, namely ligand-based and structure-based. The optimized proteins (PDB:) is made to bind with the ligand using the Autodock 4.2 for docking analysis. The binding energy was recorded. The proteins which used for analysis were downloaded from RCSB PDB database in pdb format. [18]

PHARMACOKINETIC ANALYSIS

Molinspiration were used to determine the molecular properties of the ligand molecules and helps in the process of ligand molecule like conversion into SMILES, normalization of molecules [19].

Molinspiration cheminformatics software uses the canonical smiles of the respective ligand molecule for determination of the chemical property and the bioactivity score.

PREDICTION OF BIO ACTIVITY SCORE

Molinspiration software is a freeware which is utilized in Predicting the bio activity score of the ligand that helps to understand about quick and accurate model for physio chemical, pharmacokinetic characteristics and drug likeness, etc. These characteristics are obtained for the ligand by uploading the canonical SMILES to the Molinspiration software. The Molinspiration bio activity score of brucine was evaluated. [20]

PHARMOCOKINETIC STUDY BY SWISS ADME:

SWISS ADME is computational model used to predict pharmacokinetic properties of the ligand molecule by drawing the structure of the ligand molecule which is taken for the analysis in the pre ADMET web-based application. The parameter which is going to investigated by the SWISS ADME is Human intestinal absorption and the bio availability of the ligand is also analysed by this software. Using this graphical RADAR plot is plotted. The pharmacokinetic study result of brucine by SWISS ADME is shown.[21]

In vivo STUDIES:

The test drug is purchased from Sigma Aldrich, India. The test drug is solubilised in the water and administered in respective dose. The alternative animal model issued to study anticonvulsant property of the brucine. Now a days zebrafish acts as the appropriate alternative animal model. This step is carried out after getting proper approval from institutional animal ethical committee (IAEC), totally thirty-six fishes were from Kolathur in Tamil Nadu, India [Table -1].

ZEBRAFISH HUSBANDARY

The adult zebrafish were procured from the nearby vendor and allowed for a week in the laboratory environment to acclimatized and the 12hrs cycle is maintained. The experimental procedure is carried out in the light period. Before starting the experiment, the subject is moved to the procedure room for at least 30 min to get used the lab condition. Animals were divided into six groups each group contains six fishes which used to evaluated for anticonvulsant property of the test drug brucine. The level of pain generated and animals used in this study is kept low as possible

Table 1: Animal Grouping for *in-vivo* studies

Group No	Group	Fish category	Treatment	Number of animals	Number of days
I	Normal Control	-	-	6	14
II	Disease control (PTZ)	Convulsant- induced fish	PTZ (0.9 mg)	6	14
III	Standard (PHT)	Convulsant- induced fish	Phenytoin (34 mg)	6	14
IV	Brucine (Low dose)	Convulsant- induced fish	Brucine (1 mg in 100 ml of water)	6	14
V	Brucine (high dose)	Convulsant- induced fish	Brucine (1 mg in 50 ml of water)	6	14

INDUCTION

Animals are immobilized on wet sponge in the experimental table for 10-15 seconds before starting the experiment with the help of lignocaine which acts as an anesthetizing agent and the behavioural change of the animal is observed by moving the anesthetized animal to the experimental table. Animal is Pre-treated with 500 ml of water filled in the beaker. Water in the beaker is maintained nearly with the temperature as the experimental room condition. 0.9 mg of PTZ is dissolved in 0.1 ml of water is taken as stock solution from that 10µl of solution is given to the animal using microlitre pipette for inducing convulsion. The different stages of convulsion were recorded with camera. The onset and duration timing of convulsion were observed.[22]

TREATMENT OF TEST DRUG BRUCINE

Test drug is given via oral route to the fish for 14 days at a dose 20µl to all fishes. The stock solution was prepared in the concentration of 1mg in 100ml water and 1mg in 50ml water. The control animal received vehicle. Using PTZ seizure is induced for fishes just before 30 min for evaluating the timing of maximal anti-convulsant activity. The video recording of different stages of seizure were compared for observing the maximal convulsant time.[23]

TREATMENT OF STANDARD DRUG PHENYTOIN

Phenytoin tablet was purchased from nearby pharmacy. Tablets are powdered using mortar and pestle and 34 mg of phenytoin is weighed and dissolved in 10ml of distilled water for stock solution. 10µl of solution is taken using microlitre pipette and given to the animals in the oral route for 14 days and at the last day seizure is induced using PTZ just before 30 min for evaluating the maximal anti-convulsant activity. The video recording of different stages of seizure were collected for observing the maximal convulsant time.

BEHAVIOUR ASSESMENT

Seizure test was performed in the tank with the dimension of 15cm*11cm*4cm. first, zebrafish were grouped into six for the experiments, each group receives the PTZ aqueous solution. The fish tank was covered with the black box to prevent them from the external signs which may disturb or lead them to falter during the test. seizure intensity (S1, S2, S3) was scored using previously established scoring method. Using stop watch the onset timing of different stages of seizure was recorded. After the third stage of convulsion (III), normally fish dies and the test terminated. MS222 is used as the anaesthetic agent if any fish is alive at the end of the experiment. The video recording onset and duration of convulsion were collected for observing the maximal convulsant time and the graph was plotted for standard, test drug and negative control for convulsion latency. [24]

RESULT

***In silico* DOCKING RESULTS:**

In silico docking studies was done. Receptors were downloaded from the RCSB PDB database which were taken as the target for anti-convulsion action. The results of molecular docking study are obtained by binding of test drug brucine with the receptor. By comparing the binding scores of the test drug brucine. It has the higher affinity with the JNK3 receptor comparing to receptors. JNK3 receptor has taken as target to evaluate the anti-convulsant activity. Absorption, distribution, metabolism, excretion and BBB penetration of brucine is indicated by pinkish region in a graph plot. which shows that brucine has most enhanced pharmacokinetic property. It also has the drug likeness property which shows zero violations for Lipinski's criteria.

S. No	Proteins	PDB ID	Binding energy of brucine	Binding energy of phenytoin
1	Factor inhibiting HIF-1 alpha	1h2n	-9.63	-9.32
2	Caspase 3 receptor	1pau	-7.46	-5.28
3	Estrogen receptor	2qtu	-10.01	-9.99
4	Human mitochondrial aldehyde dehydrogenase	2vle	-9.90	-8.38
5	JNK3	2waj	-11.56	-8.11
6	HDAC	3f07	-7.69	-6.62
7	MAPK 14	3u8w	-9.31	-7.45
8	GABA	4cof	-7.99	-7.85
9	AChE	4ey7	-10.66	-8.20

Table 2: *In silico* docking results of Brucine and Phenytoin

Figure 1: 2D & 3D interactions of Brucine with JNK Receptor

Figure 2: 2D & 3D interactions of Phenytoin with JNK Receptor

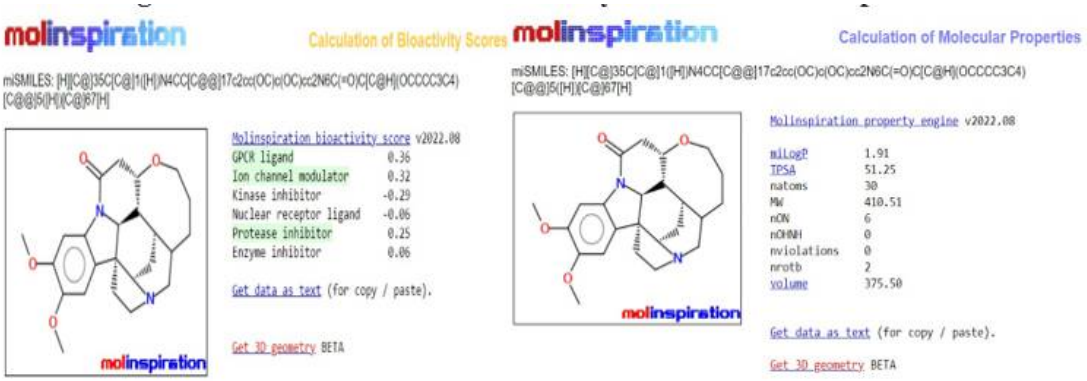

Figure 3: Bioactivity score and Molinspiration properties of Brucine

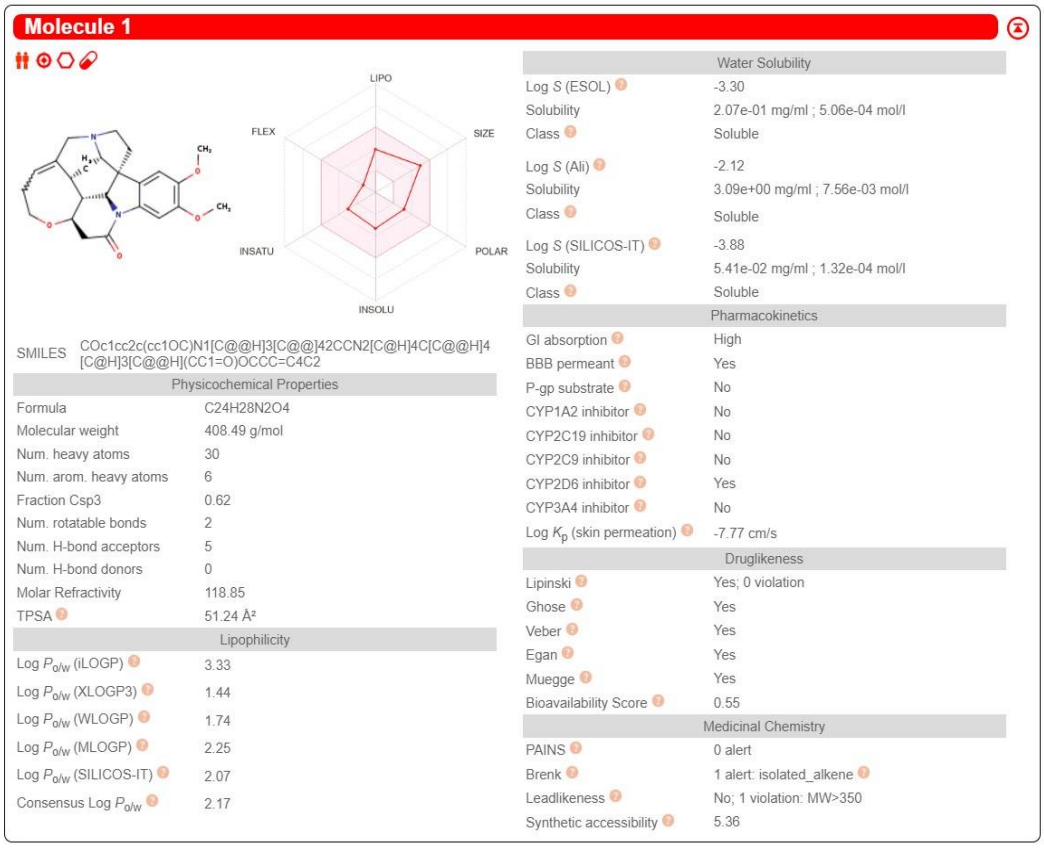

Figure 4: Pharmacokinetic property of Brucine using Swiss ADME tool

***In vivo* studies**

Graph is plotted for onset and duration of convulsion which shows the three stages of convulsion (S1, S2, S3) recorded in seconds for the period of one minute. In that it shows the convulsion latency of the fish is reduced when compared with negative control and standard group. On comparing the low and high dose of brucine with standard drug phenytoin it shows that the three phases of convulsion (S1, S2, S3) time is reduced in low and high dose of brucine when compared with phenytoin.

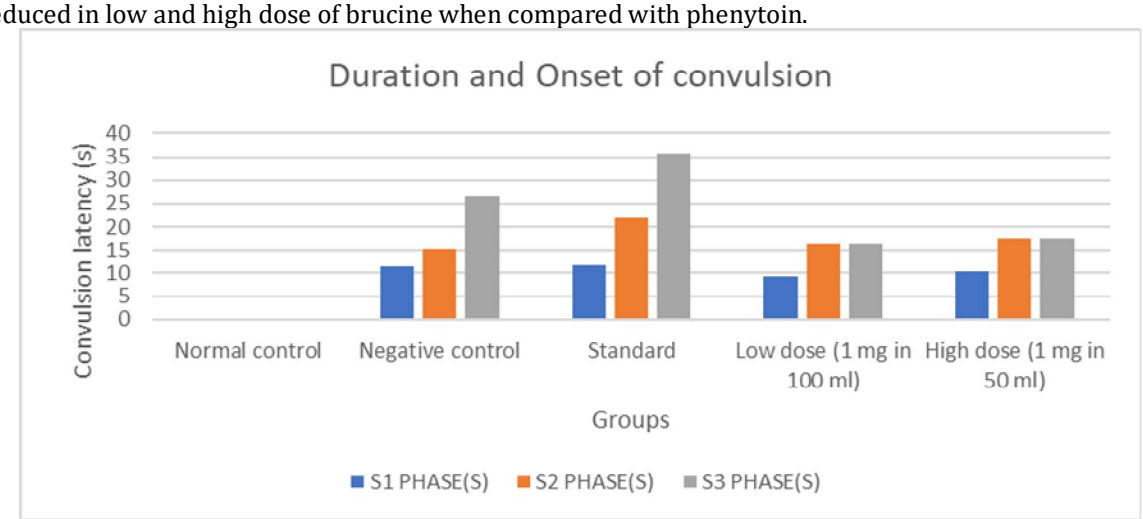

Figure 5: Effect on Brucine on duration and Onset of convulsion

Table: 3 Onset and Duration of Convulsion in Treatment groups

GROUP	S1 PHASE(S)	S2 PHASE(S)	S3 PHASE(S)
Normal control	0	0	0
Negative control	11.50	15.43	26.71
Standard	11.67	21.84	35.73
Low dose (1 mg in 100 ml)	9.36	16.31	21.39
High dose (1 mg in 50 ml)	10.46	17.58	17.58

DISCUSSION

In investigation on epilepsy, zebrafish are commonly used as model organisms. Animal exposure pentylene-tetrazole (PTZ) is currently the most accurate model for convulsions in zebrafish. Similar to PTZ, other proconvulsant substances like picrotoxin, kainic acid, or pilocarpine are less commonly utilized but nevertheless used more frequently in larvae than in adult zebrafish. In silico docking studies was done for brucine with receptors Factor inhibiting HIF-1 alpha, Caspase 3 receptor, Estrogen receptor, Human mitochondrial aldehyde dehydrogenase, JNK3, HDAC, MAPK 14, GABA, ACHE. Receptors were downloaded from the RCSB PDB database which were taken as the target for anti-convulsion action. As per, molecular docking study for brucine is conducted. By comparing the binding scores of the test drug brucine. It has the higher binding affinity with the JNK3 receptor than the other receptors. JNK3 receptor has been taken as the target to evaluate the anti-convulsant activity. Brucine possesses enhanced pharmacokinetic property and BBB penetration which is indicated by the pinkish region inside a graphical plot. It also has the drug likeness property which shows zero violations for Lipinski's criteria. Using the data obtained from the recordings of onset and duration of convulsion. Graph has been plotted with three stages of convulsion (S1, S2, S3) in sec. the convulsion latency is evaluated between the high dose and low dose of brucine. It shows that the brucine reduces the convulsion latency in (S2 and S3) than the standard drug phenytoin.

REFERENCES

1. E. Beghi, G. Giussani (2018). Aging and the Epidemiology of Epilepsy, Neuroendocrinology 51; 216–223. https://doi.org/10.1159/000493484.
2. W. Lasoñ, M. Chlebicka, K. Rejdak (2013). Research advances in basic mechanisms of seizures and antiepileptic drug action, Pharmacological Reports 65; 787–801. https://doi.org/10.1016/s1734-1140(13)71060-0.
3. M. Pieróg, K. Socała, U. Doboszewska, E. Wyska, L. Guz, A. Szopa, A. Serefko, E. Poleszak, P. Wlaź (2021). Effects of new antiseizure drugs on seizure activity and anxiety-like behavior in adult zebrafish, Toxicology and Applied Pharmacology 427 (2021). https://doi.org/10.1016/j.taap.2021.115655.
4. Y. Bozzi, M. Dunleavy, D.C. Henshall (2011). Cell signaling underlying epileptic behavior, Frontiers in Behavioral Neuroscience 5; 1–11. https://doi.org/10.3389/fnbeh.2011.00045.
5. C.A. Reid, S.F. Berkovic, S. Petrou (2009). Mechanisms of human inherited epilepsies, Progress in Neurobiology 87; 41–57. https://doi.org/10.1016/j.pneurobio.2008.09.016.
6. Annamaria Vezzani (2014). Epilepsy and Inflammation in the Brain, Epilepsy Current 14; 3–7.
7. A. Vezzani, J. French, T. Bartfai, T.Z. Baram (2011). The role of inflammation in epilepsy, Nature Reviews Neurology 7; 31–40. https://doi.org/10.1038/nrneurol.2010.178.
8. A. Subramanian, T. Tamilanban, M. Sekar, M.Y. Begum, A. Atiya, G. Ramachawolran, L.S. Wong, V. Subramaniyan, S.H. Gan, N.N.I. Mat Rani, Y.S. Wu, S. V. Chinni, S. Fuloria, N.K. Fuloria (2023). Neuroprotective potential of Marsilea quadrifolia Linn against monosodium glutamate-induced excitotoxicity in rats, Front Pharmacol 14. https://doi.org/10.3389/fphar.2023.1212376.
9. R.L. Macdonald, K.M. Kelly (1993). Antiepileptic Drug Mechanisms of Action, Epilepsia 34; S1–S8. https://doi.org/10.1111/j.1528-1157.1993.tb05918.x.
10. J. Spanoghe, L.E. Larsen, E. Craey, S. Manzella, A. Van Dycke, P. Boon, R (2021). Raedt The signaling pathways involved in the anticonvulsive effects of the adenosine a1 receptor, International Journal of Molecular Sciences 22; 1–26 https://doi.org/10.3390/ijms22010320.
11. P. Singh, R.K. Singh (2020). Synthesis of Hydrazone Derivatives and In-silico docking studies against JNK protein to assess anticonvulsant activity of synthesized derivatives, Journal of Pharmaceutical Sciences and Research 12; 770–779.
12. N. Takeshi (1977). NII-Electronic Library Service, Chemical Pharmaceutical Bulletin 57; 364–370.
13. L. Lu, R. Huang, Y. Wu, J.M. Jin, H.Z. Chen, L.J. Zhang, X. Luan (2020). Brucine: A Review of Phytochemistry, Pharmacology, and Toxicology, Frontiers in Pharmacology 11; 1–6. https://doi.org/10.3389/fphar.2020.00377.
14. M. Bello, M. Martínez-Archundia, J. Correa-Basurto (2013). Automated docking for novel drug discovery, Expert Opinion on Drug Discovery 8; 821–834. https://doi.org/10.1517 /17460441. 2013.794780.
15. P. Goldsmith (2004). Zebrafish as a pharmacological tool: The how, why and when, Current Opinion in Pharmacology 4; 504–512. https://doi.org/10.1016/j.coph.2004.04.005.
16. M.H. Abdallah, A.S. Abu Lila, R. Unissa, H.S. Elsewedy, H.A. Elghamry, M.S. Soliman (2021). Preparation, characterization and evaluation of anti-inflammatory and anti-nociceptive effects of brucine-loaded nanoemulgel, Colloids and Surfaces B: Biointerfaces 205; 111868. https://doi.org/10.1016/ j.colsurfb.2021.111868.
17. K. Lado, L. Sube, J. Daniel, W. Lako, C. Stephen, G. Lumori, J.P. Yengkopiong, J. Augustino, M. Utong, S.A. Binyason, Y. Samuel, L. Ngerja, M. Kalisto Moilinga, T.F. Lado, A.H. Kheiralla (2020). Diversity and distribution of medicinal plants in the republic of South Sudan, World Journal of Advanced Research and Reviews; 2581–9615. https://doi.org/10.30574/wjarr.

18. Scior T, Bender A, Tresadern G, Medina-Franco JL, Martínez-Mayorga K, Langer T, Cuanalo-Contreras K, Agrafiotis DK. (2012). Recognizing pitfalls in virtual screening: a critical review. J Chem Inf Model.23;52(4):867-81. doi: 10.1021/ci200528d. Epub 2012 Apr 6. PMID: 22435959.
19. Prabakaran M (2022). Biological Potential of Thiazolidinedione Derivatives: A Review, J Pharm Negat Results; 4874–4887. https://doi.org/10.47750/pnr.2022.13.S08.636.
20. A.G.A. El-Helby, R.R.A. Ayyad, M.F. Zayed, H.S. Abulkhair, H. Elkady, K. El-Adl (2019). Design, synthesis, in silico ADMET profile and GABA-A docking of novel phthalazines as potent anticonvulsants, Archiv Der Pharmazie 352. https://doi.org/10.1002/ardp.201800387.
21. Y. Wang, J. Xing, Y. Xu, N. Zhou, J. Peng, Z. Xiong, X. Liu, X. Luo, C. Luo, K. Chen, M. Zheng, H. Jiang (2015). In silico ADME/T modelling for rational drug design, Quarterly Reviews of Biophysics 48 488–515. https://doi.org/10.1017/S0033583515000190.
22. E. Samokhina, A. Samokhin (2018). Neuropathological profile of the pentylenetetrazol (PTZ) kindling model, International Journal of Neuroscience 128; 1086–1096. https://doi.org/10.1080/ 00207454.2018.1481064.
23. X.K. Deng, W. Yin, W.D. Li, F.Z. Yin, X.Y. Lu, X.C. Zhang, Z.C. Hua, B.C. Cai (2006). The anti-tumor effects of alkaloids from the seeds of *Strychnos nuxvomica* on HepG2 cells and its possible mechanism, Journal of Ethnopharmacology 106; 179–186. https://doi.org/10.1016/j.jep.2005.12.021.
24. C. Collymore, A. Tolwani, C. Lieggi, S. Rasmussen (2014). Efficacy and safety of 5 anesthetics in adult zebrafish (Danio rerio), Journal of the American Association for Laboratory Animal Science 53 198–203.