Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
4(2), 2015, 140-147

ISSN 2321 – 0958

Original Research Article

ANTICONVULSANT PROFILE OF COMBINATION OF *ANNONA SQUAMOSA, NARDOSTACHYS JATAMANSI AND BACOPA MONNIERI* AND EPI CAPS IN MICE

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Abstract: Several studies have indicated the role of *Annona squamosa*, *Nardostachys jatamansi*, *Bacopa monnieri* for its anti convulsant effects individually. Thus a combination of these extracts along with a marketed formulation Epi caps was evaluated for its anti convulsant potency. Acute Oral Toxicity was performed as per OECD 423 and this combination was found to be safe upto 2000mg/kg. The combination of extracts was given in 100mg/kg and 200mg/kg for 14 days. The anti convulsant potency of these drugs was evaluated using Pentylenetetrazole and Maximal electroshock models for epilepsy in mice. The results evidently suggest that the combination of extracts as well as Epi caps were able to control the extent and severity of seizures in PTZ and MES models.

Keywords: *Nardostachys jatamansi*, *Annona squamosa*, *Bacopa monnieri*, Anti convulsant, Pentylenetetrazole, Maximal Electroshock, Epi caps

Introduction:

Epilepsy is a common neurological disorder precipitated by recurring episodes of seizures, one of the world's oldest recognized disease and a public health concern affecting millions of people around the world prevailing in both the sexes as well as children[1]. There is an increase in the use of complementary and alternative medicines along with or without conventional medications for management of epilepsy. Herbal medicines are found to be effective in several cases and this forms an important part of Complementary and alternative medicines[2]. There are several herbs that have been proven individually for its effect on seizures. *Nardostachys jatamansi*(NJ) (Valerianaceae) has been used for management of epilepsy in Ayurveda[3]. It helps to promote physical and mental health, augment resistance of the body against disease, and has shown potent antioxidant activity. It also shows a marked tranquilizing activity, as well as, hypotensive, hypolipidemic anti-ischemic, anti-arrythmic, hepatoprotective, anticonvulsant, and neuroprotective activities[4]. *Bacopa monnieri* Linn. (BM) belonging to family

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Received on: May 2015
Accepted after revision: May 2015
Downloaded from: www.johronline.com

Scrophulariaceae is an important constituent of several polyherbal formulations like Saraswatarishta, BrahmiGhrita and Mentat which are widely used for various neurocognitive[5]. Studies have shown that it exhibits its anti convulsant properties via GABA receptors[6]. *Annona squamosa* Linn. (AS) belonging to family Annonaceae, widely known as custard apple have been used by few traditional healers in case of epileptic attacks. It is reported to have varied therapeutic activities like anti diabetic, anti hyperlipidemic, expectorant, anti-cancer and insecticidal properties. Ethanolic extract of its leaves is reported for its anti convulsant activity[7]. Thus a polyherbal formulation consisting of Jatamansi, Sitaphal leaves and Brahmi was prepared to evaluate its efficacy on seizures embarking additive effect of these drugs.

Along with this combination of extracts, a marketed formulation called Epi caps was also evaluated for its use as anti convulsant. It has been prescribed by local Ayurvedic and Unani practioners for epilepsy. It has varied combination of dried extracts of Aloe vera, *Boswellia serrata, Centella asiatica, Evolvulus alsinoides*. Thus, the efficacy of this formulation was also determined for management of epilepsy and a comparison of this formulation along with the combination of extracts of AS, NJ and BM was evaluated. The combination of extracts is referred to as Test 1 (100mg/kg) and Test 2 (200mg/kg).

Materials and Methods

Drugs and Chemicals: Pentylenetetrazole was obtained from SRL Chemicals. *Bacopa monnieri* extract was received as a gift sample from Sami Labs, Bangalore. Phenytoin was also received as a gift sample from Crest Healthcare, Vadodara. Epi caps was procured from a local ayurvedic store.

Animals: Albino Swiss mice weighing 22-28g were procured from Bharat Serums Pvt Ltd., Thane and housed in polypropylene cages at 22-25^{o} C under a 12 hour light/ dark cycle. The mice were fed with standard pellets and water *ad libitum.* The animals were housed under these conditions and allowed for acclimatization for 7 days. The experiments were performed after obtaining approval from Institutional Animal Ethics Committee (CPCSEA/ IAEC/BNCP/P-17/2014). Animals were divided into 6 groups for the study. Each group consisted of 6 animals. Group A Control group (0.5% CMC). Group B: Phenytoin (20mg/kg), Group C Test 1(100mg/kg), Group D Test 2(200 mg/kg), and Group E Epi caps (500mg /capsule).

Extraction procedure and preparation of drug: The leaves of AS were collected from Vasai, Palghar district, Maharashtra, India in the month of May and were shade dried. Jatamansi dried rhizomes were obtained from Pharmacognosy Laboratory of Dr. Bhanuben Nanavati College of Pharmacy. The leaves and rhizomes were identified and authenticated at Department of Botany, Mithibai College of Arts, Chauhan Institute of Science and AmrutbenJivanlal College of Commerce and Economics, Mumbai for reference Voucher MIT 009 (AS) and MIT 0069 (NJ). The dried plant materials were powdered and soxhlet extraction using 70% ethanol and 95% ethanol was performed for AS leaves and NJ roots respectively. After complete extraction, the extracts were dried using rotary vacuum evaporator. A suspension of these extracts was prepared in 0.5% CMC in the ratio of 1:1:1. The dose for administration was calculated and expressed as mg/kg of body weight.

Acute Toxicity and gross behavioral changes: An acute toxicity study was performed for the combination and Epi caps as per OECD (Organization for Economic Cooperation and Development) Guidelines for Acute Oral Toxicity 423 for a period of 14 days. Three animals were randomly selected, marked to permit individual identification, and kept in

their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were fasted for 4 hours prior to oral dosing of the test compounds. Food was further withheld for 1 hour after dosing. .These animals were kept under close supervision with a regular supply of food and water *ad libitum* and were checked for probable signs of toxicity for the first 1, 4, 12 and 24 hours. The weight of all these animals was noted regularly on day to day basis. The animals were monitored for visual observations pertaining to skin and fur, eyes and mucous membrane, respiratory, circulatory, and autonomic and central nervous system, somatic activity and behavioral patterns. At the end of the study, blood was collected from these animals for hematological and biochemical assays and further the animals were euthanized on the last day of the experiment[8].

Pentylenetetrazole (PTZ) Model to determine anticonvulsant potency: Phenytoin, Test 1 and Test 2, Epi caps were administered orally to the specific group of mice. 60 mins after oral administration of these test compounds for a period of 14 days, 60mg/kg of PTZ (in saline) was injected intraperitoneally. Each mouse was placed individually in plastic cages to record observations. The onset time and duration of each seizure was noted for all the groups. The animals were considered to be protected against seizures when no convulsion or mortality occurred within 30 mins[9]. Animals were divided into 6 groups for the study. Each group consisted of 6 animals. Group A: Control group (0.5% CMC). Group B: Phenytoin (20mg/kg), Group C Test 1(100mg/kg), Group D Test 2(200 mg/kg), and Group E Epi caps (500mg /capsule).

Maximal Electroshock (MES) Model to determine anticonvulsant potency: MES model is preferred over other complex models as it facilitates screening of anti convulsants drugs and its development in relatively short span of time and for high number of test compounds[10]. MES test is started after 60 mins of oral administration of Phenytoin, Test 1, Test 2 and Epi caps. Ear clip electrodes were used to deliver stimuli with the intensity of 12 mA, 50 Hz for 0.2 s to each group of mice. The duration of each of the following phase was recorded in observations: Tonic Flexion, Tonic Extension, Clonus and Stupor. The suppression of each of the phases indicates the efficacy of anti-epileptics. The control group characteristically showed these phases[11, 12].

Results

Acute Toxicity and gross behavioral changes: The combination of extracts did not lead to any gross behavioural changes such as increase or decrease in motor activity, weight loss, tremors, convulsions, muscle spasms, loss of righting reflex, spasticity, sedation, muscle relaxation, ataxia, watery diarrhea, lacrimation and salivation during the study. The oral dose of this combination was non-lethal at 2000mg/kg.

Statistical Analysis: The data was analyzed using One way ANOVA followed by Tukey Post hoc test using Graph Pad In Stat 3.0 software. The time of each seizure is described in all the tables below as Mean ± SEM in seconds.

Pentyleneterazole model: Pentylenetetrazole (60mg/kg) produced generalized clonic and hind limb tonic seizures in the animals. Phenytoin (20mg/kg) offered highly significant protection against seizures by causing an immense delay in the onset time as well decreasing the duration of each seizure by several manifolds when compared with control. Test1, Test 2 and Epi caps exhibited significant protection against onset time of seizures. Seizure threshold also increased to a great extent. Thus, highly significant reduction in the mean seizure duration for Test 1, Test 2 and Epi caps in comparison to negative control was observed.

Table 1: Effect of Phenytoin, Test 1, Test 2 and Epi caps on PTZ model:

Group	**Onset Time (secs)**	**Duration (secs)**
Control	191.83 ± 11.36	301.67 ± 11.38
Phenytoin	446.17 ± 3.33 ***	7.83 ± 7.83 ***
Test 1 (100mg/kg)	302.16 ± 19.14 *	144.17 ± 13.84 ***
Test 2 (200 mg/kg)	317.5 ± 24.62 **	129.83 ± 11.43 ***
Epi caps (500mg/capsule)	337.16 ± 24.36 **	94.83 ± 6.51 ***

In the table above, * indicates significance in comparison to control. *** P < 0.0001 ** P < 0.01 * P < 0.05

Fig 1: Effect of Phenytoin, Test 1, Test 2 and Epi caps on mean seizure onset time PTZ model:

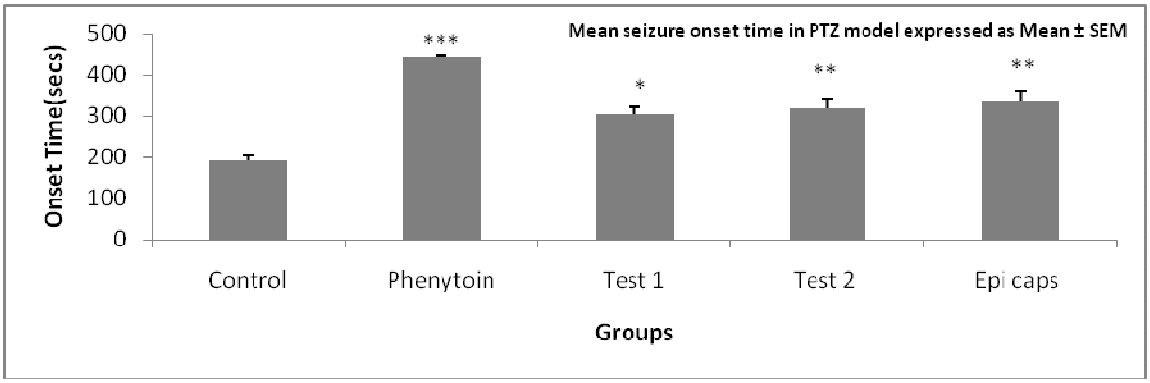
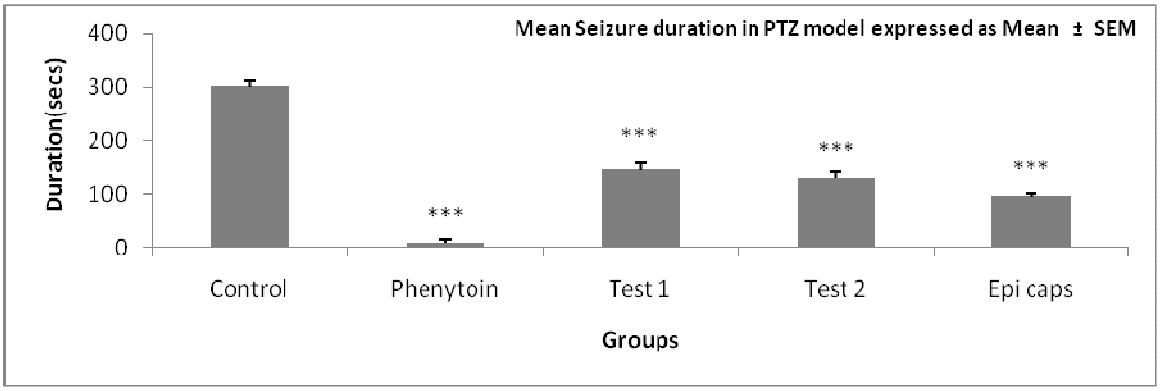


Fig 2: Effect of Phenytoin, Test 1, Test 2 and Epi caps on Mean seizure duration in PTZ model:



Maximal Electroshock Model: The combination of extracts as well as Epi caps showed significant reduction in tonic hindlimb flexion and extension, as well as in clonus duration. The Phenytoin group exhibited 83.33% protection against MES induced seizures. However, highly significant improvement was observed in stupor duration.

Table 2: Effect of Phenytoin, Test 1, Test 2 and Epi caps on MES model:

Group	Tonic Hindlimb Flexion (secs)	Tonic Hindlimb Extension (secs)	Clonus Duration (secs)	Stupor duration (secs)
Control	13.5 ± 1.38	11.33 ± 1.89	26.16 ± 3.30	221.83 ± 26
Phenytoin	0.33 ± 0.84 ***	0.67 ± .67 ***	1.33 ± 1.33 ***	17.83 ± 17.83 ***
Test 1	7.5 ± 0.99**	6.17 ± 1.30 *	12.83 ± 1.40 *	127.33 ± 11.33 ***
Test 2	7.16 ± 1.27 **	5.5 ± 0.71 **	13.67 ± 2.23 *	104.83 ± 11.30 ***
Epi caps	6.67 ± 1.11 ***	6 ± 0.89 *	12.17 ± 1.95 *	95.67 ± 6.75 ***

In the table above, * indicates significance in comparison to control. *** P < 0.0001 ** P < 0.01 * P < 0.05

Fig 3: Effect of Phenytoin, Test 1, Test 2 and Epi caps on Tonic hind limb flexion duration in MES model:

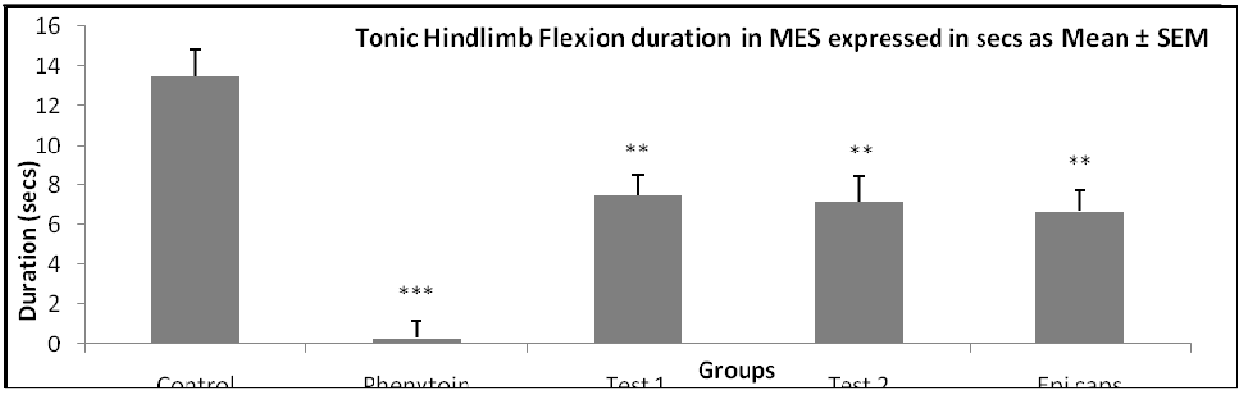


Fig 4: Effect of Phenytoin, Test 1, Test 2 and Epi caps on Tonic hindlimb extension duration in MES model:

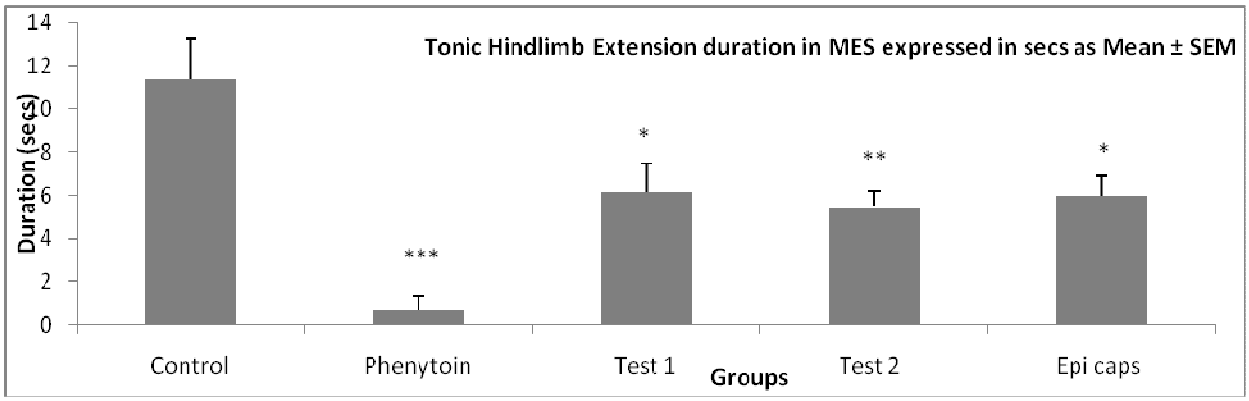


Fig 5: Effect of Phenytoin, Test 1, Test 2 and Epi caps on Clonus duration in MES model:

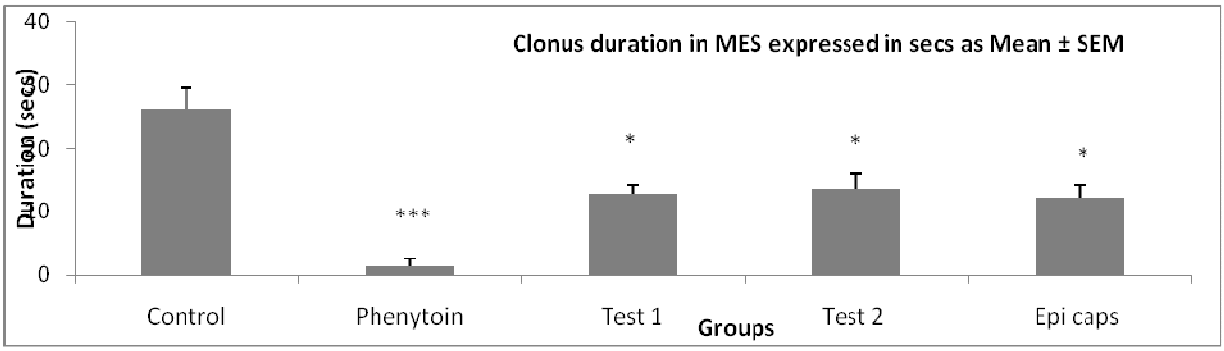
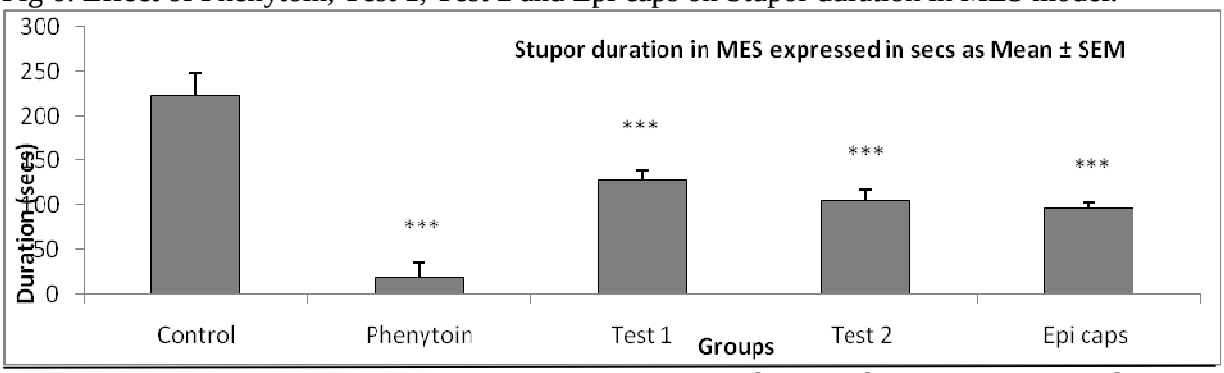


Fig 6: Effect of Phenytoin, Test 1, Test 2 and Epi caps on Stupor duration in MES model:



Discussion:

Epilepsy continues to be a major public health concern worldwide. Several times in patients suffering from refractory partial or generalized tonic clonic seizures, polypharmacy is often recommended for nearly 30% of the patients[13]. The available anti-epileptic drugs treat symptoms rather than the disease itself. This has garnered interest in traditional approaches which includes herbal drugs and formulation. These are regarded as safe for extended usage for management of epilepsy[14]. Similarly, the available drugs for epilepsy suggest that there is no specific satisfactory to meet the needs of the therapy. It is known in the case of epilepsy that the major cause is the imbalance occurring between excitatory and inhibitory neurotransmission in the brain[15]. Thus, Experimental animal models are used to provide significant information as well as they clearly indicate safety and efficacy of drugs. In case of epilepsy, PTZ and MES induced seizures are widely performed and conventionally accepted for screening of anti convulsant drugs[16].

It is known that seizures caused using MES model, are able to induce tonic extension by altering the functions of voltage gated ion channels or by accelerating glutaminergic excitation mediated by N-Methyl-D-Aspartame receptor[17]. Membrane excitability, sub threshold electrical excitability, neurotransmitter release epileptic form discharge are all regulated by Na^+, Ca^{2+} and K^+ channels[18]. Thus these seizures induced by MES can be inhibited by blocking glutminergic excitation and voltage gated ion channels[19].

PTZ acts by modulating the GABA function. PTZ is a chemoconvulsant which acts by binding to a picrotoxin site on GABA receptor to decelerate chloride channel functioning[20]. The extracts were able to antagonize PTZ mediated seizures. Hence, it can be estimated that this combination of extracts and Epi caps might have an effect on GABAnergic transmission.

In traditional system of medicine, drugs are administered in crude form. Hence, the herbal approach is slow acting when compared to modern day anti-epileptic drug therapy. Thus,

these extracts were administered for a period of 14 days. In this study, we have demonstrated the additive effect and of these herbs and its benefits. The aim of these polyherbal extracts is that to propose such combinations that can act through varied mechanisms, reduced dose and better efficacy and lesser toxicity than monotherapy. This combination did not show any signs of neurotoxicity. In this study, Test 1 i.e. 100mg/kg is exhibiting similar efficacy when compared to Test 2 which is 200mg/kg. Thus, lower dose can be used for elevating seizure thresholds. Epi caps too have shown significant protection against seizures when compared to control group which were given only vehicle in both the animal models.

Conclusion:

The results evidently suggest that the combination of extracts of *Anonna squamosa, Bacopa monnieri and Nardostachys jatamansi* as well as Epi caps control the extent and severity of seizures in PTZ and MES models. Since the extracts and Epi caps are found to be showing protection against seizures, it can be assumed that they act on both the mechanisms, i.e. altering the functioning of Voltage gated channels and glutaminergic excitability as well as acting as an agonist on the $GABA_A$ receptor. However, further studies are required to determine and validate the exact mechanism of action and the active constituents of these plants responsible for showing action.

Acknowledgement:

The author is thankful to Crest Healthcare, Vadodara and Sami Labs, Bangalore for providing free gift samples. The authors are indebted to Dr. BhanubenNanavati College of Pharmacy for providing the facilities required to carry out this study.

References:

1. WHO, Epilepsy, Fact Sheet N^{o}999, May 2015 (http://www.who.int/mediacentre/factsheets/fs999/en/).
2. Samuels N., Finkelstein Y, Singer SR, Oberbaum M. Herbal medicine and epilepsy: proconvulsive effects and interactions with antiepileptic drugs. Epilepsia. 2008 Mar; 49(3):373-80
3. Rao VS, Rao A, Karanth KS. Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats. J Ethnopharmacol. 2005 Dec 1; 102(3):351-6.
4. Lyle N, Bhattacharya D, Sur TK, Munshi S, Paul S, Chatterjee S, Stress modulating antioxidant effects of *Nardostaychys jatamansi*. Indian J Biochem Biophys 2009; 46:93-8.
5. Shital A. Giramkar, Omkar P. Kulkarni, Suresh D. Jagtap, Aniket A. Kuvalekar, Sourav Mukherjee, Rutika R. Jagtap, Ajay R. Wagh, Deepti D. Bandawane, Sonali S. Nipate, Anticonvulsant potential of commonly practiced formulations of Brahmi (Bacopamonnieri Linn.) in Wistar rats, Journal of Pharmacy Research, Volume 7, Issue 9, September 2013, Pages 787-791
6. Mathew J, Soman S, Sadanandan J, Paulose CS. Decreased GABA receptor in the striatum and spatial recognition memory deficit in epileptic rats: effect of Bacopamonnieri and bacoside-A. J.Ethnopharmacol. 2010 Jul 20; 130(2)
7. Porwal M, Sharma K. Evaluation of Anticonvulsant activity of *Annonasquamosa*Linn leaves in mice. J. of Cell and Tissue Research, 2011, 11(2): 2879-2884.
8. OECD. Guidance document on Acute Oral Toxicity. Environmental Health and safety monograph series on safety and assessment No. 24. 2001.
9. H.Gerhard Vogel (Ed), *Drug Discovery and Evaluation:Pharmacological Assays*, Second Edition, Springer-Verlag, Berlin Heidelberg, Germany 2002; pp. 422-423.
10. Wolfgang Löscher, Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs, Seizure, Volume 20, Issue 5, June 2011, 359-368

11. Swinyard EA., Electrically induced convulsions. In Purpura, D., Penry, J., Tower,D., Woodbury, D., Walter, R. (Eds), Experimental Models of Epilepsy. Raven Press, New York, pp. 434-456.
12. H.Gerhard Vogel (Ed), *Drug Discovery and Evaluation:Pharmacological Assays*, Second Edition, Springer-Verlag, Berlin Heidelberg, Germany 2002; pp. 487.
13. Pellock, J.M., 1995. Antiepileptic drug-therapy in the United-States—a review of clinical-studies and unmet needs. Neurology 45, S17–S24.
14. Singh, H.K., Dhawan, B.N., 1997. Neuropsychopharmacological effects of the AyurvedicnootropicBacopamonniera Linn. (Brahmi). Indian Journal of Pharmacology29, 359–365.
15. El-Tahir KE, Ashour MM, Al-Harbi MM. The cardiovascular actions of volatile oil of the black seeds (*Nigella sativa*) in guinea–pigs: elucidation of the mechanism(s) of action. Gen pharmacol 1993; 24:1123-1131.
16. Fisher RS. Animal models of the epilepsies. Brain Res Rev 1989; 14:245–278.
17. Patten D, Foxon GR, Martin KF, Hallwell RF. An electrophysiological study of the effects of propofol on native neuronal ligand-gated ion channels. ClinExpPharmacolPhysiol 2001; 28:451-458.
18. Stefani A, Spadoni F, Giacomini P, Lavaroni F, Bernardi G. The effects of gabapentin on different ligand- and voltage-gated currents in isolated cortical neurons. Epilepsy Res 2001; 43:239-248.
19. Subramaniam S, Rho JM, Penix L, Donevan SD, Fieldin RP, Rogawski MA. Felbamate block the N-methyl-Daspartame receptor. J PharmacolExpTher 1995; 273:878-886.
20. Deshmane DN, Gadgoli HC, Halade VG. Anticonvulsant effect of *Origanmmajorana* L., Pharmacologyonline, 2007; 1:64-78.