International Journal of Phytomedicine 7 (2015) 106-111
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antiepileptic activity of *Unmadgajakesari* – A herbomineral formulation: An experimental evaluation

Rajeeta Joseph[1] , Vijaya Pandit[2] , Asmita Wele[3], Gourav Deshmane[1]

***Corresponding author:**

Rajeeta Joseph

[1]Department of Pharmacology, B.V.D.U. Dental College and Hospital. Pune
[2]Department of Pharmacology, B.V.D.U Medical College. Pune
[3]Department of Rasashastra, B.V.D.U. College of Ayurveda, Pune

Abstract

Epilepsy is the most common chronic neurological disorder characterized by episodes of recurrent unprovoked seizures *Unmadgajakesari*(*UGK*) is a herbomineral formulation claimed to be useful in epilepsy in traditional medicine. Lack of scientific evidence of UGK for its use in epilepsy lead to the objective of the present work.

To evaluate the antiepileptic activity of Unmadgajakesari in animal models

After doing the acute toxicity study of UGK, it was evaluated for its antiepileptic activity in Maximal Electroshock(MES) and Pentylenetetrazole(PTZ) induced seizures models in albino wistar rats. For each study animals were divided into 6 groups, each group comprising of 6 animals . Group I –Normal control, Group II-Vehicle control(ghrita), Group III- Drug control(positive control). In test groups(IV-VI) UGK was administered in doses of 70, 140 and 280mg/kg orally for 8days. Antiepileptic activity was evaluated on day 1and 8.

UGK was found to be nontoxic upto dose of 2000mg/kg. Significant antiepileptic activity was observed in both the groups on 8th day of UGK administration. In the MES model, significant abolition of tonic hind limb extension was observed in dose of 280mg/kg. In PTZ model, UGK was most effective in the dose of 70mg/kg in delaying the onset and reducing the severity of clonic convulsions. No adverse effects or mortality was seen in this study.

UGK appears to have significant antiepileptic activity after repeated administration. With wide spectrum of action, this drug may be useful addition to antiepileptic agents.

Keywords: Unmadgajakesari, Antiepileptic, Gabaergic, Antioxidant

Introduction

Epilepsy is a common and chronic neurological disorder characterized by apparently unprovoked recurrent paroxysmal events or seizures that are associated with a sudden alteration in motor activity and behavior, with or without alteration in conscious awareness. The alteration in state is the result of an abnormal and excessive hyper synchronous firing within a group of epileptic neurons in the brain.[1] Epilepsy affects approximately 70 million people of all ages throughout the world. It is responsible for 1% contribution to the global burden of diseases while this contribution is 80% in the developing countries.[2]

In epilepsy, hypoactivity of GABA, which has inhibitory function, and a hyperactivity of glutamate, which acts mainly as an excitotoxic, postsynaptic excitatory neurotransmitter have been reported.[3] Glutamate, $GABA_A$ receptor and voltage activated Na^+ and Ca^{2+} channels represent the major targets of antiepileptic drugs(AEDs).[4] Carbamazepine, ethosuximide, phenobarbital, phenytoin, and valproate are the most frequently used conventional antiepileptics(AEDs). The therapeutic failure in 20-25% of patients has stimulated intensive research on novel antiepileptic drugs and so far most of them have been developed and licensed mainly as add-on treatment in patients poorly responding to conventional therapy. These are felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, tiagabine, topiramate, vigabatrin, and zonisamide.[5] Majority of antiepileptic drugs possesses more than one mechanism of action. Deckers et al. have proposed a classification of antiepileptic drugs based upon these mechanisms. First group- antiepileptics (carbamazepine, gabapentin, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, topiramate, valproate) which block sustained repetitive firing in individual neurons, by blocking voltage-dependent sodium or calcium channels. These drugs are effective against generalized tonic-clonic and partial seizures. The second group- drugs enhancing inhibitory events mediated by γ-aminobutyric acid (GABA) i.e benzodiazepines, gabapentin, phenobarbital, tiagabine, topiramate, vigabatrin, and valproate. Some of these drugs may be used in all seizure types(absence, generalized tonic-clonic, and partial seizures). The third– ethosuximide which blocks T-type calcium channels and is active against absences.[6] Zonisamide is a new broad spectrum antiepileptic drug efficient in treating refractory epilepsy by inhibiting voltage-dependent Na^+ channels and Ca^{2+} channels of T-type.[7] A separate category of drugs reduce events mediated by excitatory amino acids(glutamate) and at present

three antiepileptics meet these criteria: felbamate, phenobarbital, and topiramate.[6] Although the last decade brought the introduction of several new AEDs and progress in the pharmacotherapy of epilepsy, about 30% of patients still have seizures that continue despite taking AEDs.[8] When the monotherapy was ineffective, polytherapy treatment is usually required. Therefore, possibility of interactions between AEDs can be a considerable problem in such situations.[7] In the light of the presented data, extensive research dealing with new AEDs should be performed.

Traditional medicinal practices have remained as a component of health care system of

many societies in spite of the availability of well-established alternatives. Many people in the developing countries still rely on traditional medicine for their basic health care needs. WHO encourages the addition of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries.[9] Scientific research is needed to provide evidences for safety and efficacy of beneficial medicinal plants. Thus a need arises for new agents with greater efficacy, negligible or reduced side effects and devoid of unfavourable drug interactions unlike most antiepileptic drugs in the market today.

Unmadgajakesari (UGK) is herbomineral drug formulation especially designed for use in treatment of psychosis *(Unmad)* and epilepsy *(Apasmar)*.[10] It contains :- Minerals - *mercury, sulfur, realgar (manashila)* and Herbs – *Dhatura.inoxia (Dhatur), Acorus.calamus (Vacha), Sesbania.grandiflora (Agasti) and Bacopa.monierri (Brahmi)*.[10] The limitations of conventional AEDs and the lack of scientific evidence of *Unmadgajakesari* for its use in epilepsy accelerated the objective of the present work to assess a safe antiepileptic drug.

Materials and Methods

Drugs

All chemicals used in this study were of analytical grade. PTZ were purchased from Sigma- Aldrich, USA, Sodium valproate and Phenytoin sodium (Sun Pharma laboratories Ltd, Mumbai) Water for injection (WFI) was purchased from pharmacy shop. Raw drugs for preparation of *Unmadgajakesari* were obtained from local market.

Preparation of UGK

UGK was prepared according to the method written in textbook Rasa Chandanshu – chapter 13 titled Unmad Chikitsa using minerals - mercury, sulfur, realgar (manahshila), and herbs – Dhatura (Dhatura.inoxia-seeds), Acorus.calamus (Vacha-rhizome), Sesbania grandiflora (Agasti-leaves) and Bacopa monnieri (Brahmi-whole plant).[11] All the ingredients were identified and authenticated following the standard procedures of Ayurvedic Pharmacopoeia of India(API). Mercury, sulfur, realgar and Dhatura (seeds) were subjected to detoxification (shodhan) process. Equal quantity of detoxified Mercury and detoxified Sulphur were triturated together until it was converted into black lusterless mixture to form kajjali. Kajjali was then mixed and triturated with detoxified realgar to form a fine mixture. To this mixture was added fine powder (mesh size 60) of detoxified dhatura seeds and further triturated to form a homogenous mixture. It was then subjected to wet trituration(bhavna) for 5 hrs daily. Seven Bhavanas each of Vacha rhizome decoction , juice of Agasti leaves and juice of whole plant of brahmi was given to the mixture sequentially to form Unmadgajakesari. This mixture i.e Unmadgajakesari (UGK) was then dried completely to render it moisture free. It is administered along with cow ghee (ghrita).[10]

Animals

Animal Ethics committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA New Delhi, India . Animals (Swiss Albino mice and Wistar rats) of either sex from our breeding stock were used in this study. They were in-housed at the institute animal house in groups of six animals per cage at standard laboratory conditions at a temperature of 24 ˚C ± 1 ˚C, relative humidity of 45–55% and 12:12 h dark and light cycle. Animals had free access to standard pelleted laboratory animal diet and water *ad libitum*. The experimentation was carried out in noise free area.

Acute Toxicity Study

The acute toxicity of *UGK* was determined as per internationally accepted protocol drawn under Organisation for Economic Co-operation and Development (OECD) guideline no. 423.[12] UGK was found to be safe even at 2000mg/kg dose.

Experimental Study

Animals were divided into 6 groups, each group comprising of 6 animals. Group I – Normal control, Group II- Ghrita (vehicle control), Group III- Drug control(positive control). The therapeutic dose of *UGK* in humans is 750mg.[11] This dose was extrapolated to animal doses[13] as X dose. In test groups(IV-VI), UGK was administered in doses 70mg/kg(X-dose), 140mg/kg(2X-dose) and 280mg/kg(4X-dose) in rats along with *ghrita* (cow ghee). All the drugs were given orally for 8 days. Antiepileptic activity was evaluated on day 1and 8.

Maximal Electroshock(MES)

Seizures are induced to all the groups by using an Electro convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. Animals were observed for inhibition of tonic hind limb extension (THLE).

Pentylenetetrazole(PTZ) induced seizures- Pentylenetetrazole (PTZ) 60mg/kg, i.p was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30mins post – PTZ administration for onset, severity and abolition of convulsions.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance. Statistical analysis was done with Graph Pad Prism 5 software.

Results

In acute toxicity study UGK was found to be safe upto 2000mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity were observed. Adverse effects such as sedation , loss of righting reflex or mortality was not observed. No pathological changes were seen in liver, kidney, heart, lung and brain.

In our experimental study, Significant antiepileptic activity was observed in Maximal Electroshock (MES) and Pentylenetetrazole (PTZ) induced seizures on 8th day of UGK administration.

Maximal Electroshock Seizure

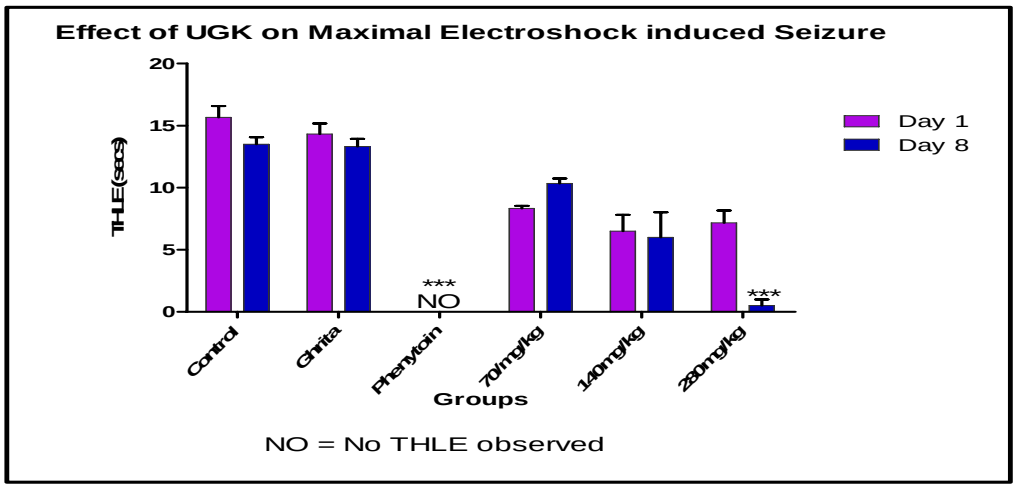

Figure 1. Effect of UGK on maximal electroshock induced seizure in rats. Each column represents mean ± SEM of number of duration of THLE. ***P<0.001 when compared to control and ghrita.

In MES induced seizure model – *UGK* in all doses decreased the duration of tonic hind limb extension (THLE) on day 1, but abolished THLE in higher dose (280mg/kg) on day 8 when compared to control groups. Phenytoin treated animals showed 100% protection against MES induced seizures whereas UGK at dose 280mg/kg offered 84% protection when compared to control group.

Pentylenetetrazole induced seizure

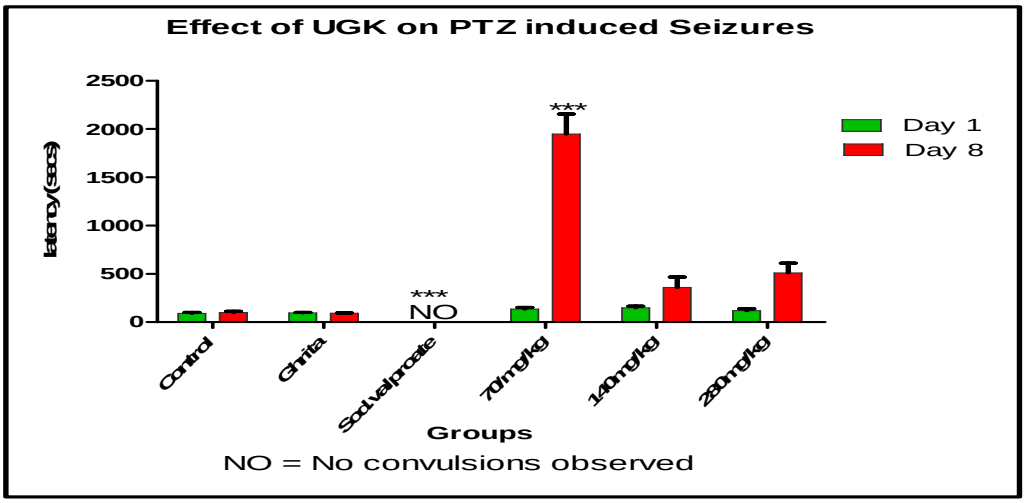

Figure 2. Effect of UGK in Pentylenetetrazole induced seizure in rats. Each column represents the mean ± SEM of latency of onset of convulsions. ***p<0.001 when compared with control and ghrita.

In the PTZ induced seizure model UGK did not have any effect on seizures on day 1 at any of the doses. But on day 8, UGK at low dose (70mg/kg) significantly($P<0.001$) increased the latency of onset of convulsions as well as reduced the severity of convulsions when compared to control group. There was no loss of righting reflex seen in all the animals of this group. Sodium valproate offered complete protection in animals on day 1 and day 8.

Discussion

Unmadgajakesari (UGK) is a herbomineral formulation; having minerals like *mercury, sulfur, realgar* and herbs like *Dhatura.inoxia* (*Dhatura*), *Acorus.calamus* (*Vacha*), *Sesbania.grandiflora* (*Agasti*) and *Bacopa.monierri* (*Brahmi*).[10] In our toxicity study UGK was found to be safe upto 2000mg/kg. No signs and symptoms of toxicity or mortality was observed. No pathological changes were seen in liver, kidney, heart, lung and brain.

UGK is used clinically to treat Psychosis and Epilepsy.[10] In the present study, antiepileptic activity of *UGK* was evaluated.

AEDs have been screened in animal models of epilepsy, often with an incomplete knowledge of their mechanism of action.[14] The most widely used *in-vivo* models to test anti-epileptic activity have been the maximal electroshock (MES) test and the pentylenetetrazol PTZ) test in normal mice/rats.[15] Hence the antiepileptic activity of *UGK* was evaluated in these models in our study.

The MES model is used to identify compounds which prevent initiation and potentiation of seizure spread corresponding to tonic-clonic seizure in humans. These can be prevented either by drugs that inhibit voltage-gated Na^+ channels such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutamatergic excitation mediated by N-methyl D-aspartate(NMDA) receptor such as felbamate.[16] Glutamate is an excitatory neurotransmitter and GABA is inhibitory neurotransmitter. So, activity of NMDA receptors can also be reduced by increasing GABA activity. *UGK* in high dose reduced the duration of tonic hind limb extension (THLE) after a single dose. But when *UGK* was given daily for 8 days, it abolished the THLE in five out of six animals thus exhibiting significant antiepileptic activity(Figure 1).

Drugs effective in PTZ induced seizure are useful in petitmal and generalized seizure in humans.[17] The convulsive effect of PTZ is via specific GABA(gamma-amino-butyric-acid) coupled chloride channels blockade. These type of seizures can also be prevented by drugs that enhance GABA activity i.e benzodiazepines . Drugs that block glutamatergic excitation mediated by NMDA receptors such as felbamate also have anticonvulsant activity against PTZ-induced seizures.[17] In this model, *UGK* in low dose, was effective in delaying the onset and reducing the severity of clonic convulsions, suggesting GABAergic activity of herbs[18] on day 8 (Figure 2). There was no increase in activity on increasing the dose. No adverse effects such as loss of righting reflex, sedation or mortality was observed.

UGK formulation contains *Kajjali* (combination of mercury and sulfur). Mercury affects Central nervous system (CNS) as it easily crosses the blood–brain barrier, gets accumulated in the brain thus affecting multiple cellular functions.[19] Mercury readily forms covalent bond with sulfur. This property accounts for most of the biological properties of the metal. It is reported that addition of Sulfur counteracts the toxicity of mercury.[20] *Kajjali* own properties as *yogavahi* (catalyst) which helps in carrying other drugs to CNS and enhance the efficacy and potency of the formulation.[21]

Manashila (realgar) is a arsenical compound advised for epilepsy in ayurvedic text[22]. Naveen et al. have reported the sedative-hypnotic activity of realgar probably by potentiating the activity of GABA[22], though the exact mechanism is unclear.

D.inoxia and *Atropa belladonna* belong to the family *Solanaceae*.[23] Antiepilepileptic activity of *D.inoxia* is not reported. Hanan et al have demonstrated the anticonvulsant activity of *Atropa belladonna* in PTZ induced seizure models. Anticonvulsant activity was attributed to presence of higher concentration of hyoscine (scopolamine) than hyoscyamine, the latter being CNS stimulant.[24] In our HPTLC studies, *D.inoxia* showed higher concentration of scopolamine(10.11μg/mg) than hyoscyamine(5.85μg/mg) suggesting its role in epilepsy. Wudayagiri Rajendra et al. have reported an increase in ACh and decrease in AChE activity in different regions of rat brain and skeletal muscles during PTZ -induced epilepsy. It is known that excessive levels of ACh in tissue can produce epileptiform activity.[25] *D.inoxia* being an anticholinergic may be useful in antagonizing excessive cholinergic activity and exerting antiepileptic activity in PTZ model.

Herbs like *Acorus, Sesbania and Bacopa* are proved to be useful in epilepsy.[18] The rhizomes of *A.calamus* have traditionally been used in the treatment of epilepsy either alone or as a component in Ayurvedic preparations, its active constituent being α and β-asarone.[26] On chronic administration, -asarone exerted good antiepileptic effect in the MES, PTZ and lithium-pilocarpine induced seizures in animals. The effects were of lesser magnitude than conventional AEDs. -asarone was also reported to increase the γ-aminobutyric acid(GABA) level and decrease the glutamate level in the brain of seizure animals.[27] Recent evidence indicates that asarone block NMDA(N-methyl-D-aspartate) receptors and thus exhibit neuroprotective activity against NMDA or glutamate induced excitotoxicity.[28] In a study by Hazra et al, *A.calamus* prevented the development of $FeCl(3)^-$induced epileptogenesis by modulating antioxidant enzymes thus exhibiting its potential of being an effective antiepileptic drug.[29]

The leaves of *S.grandiflora (Agasti)* are used in Ayurveda for the treatment of epilepsy. In a study by Kasture et al. *S.grandiflora* exhibited wide spectrum of anticonvulsant activity in MES, PTZ and strychnine induced seizures by increasing levels of GABA in brain.[30] Leaves of *S.grandiflora* have been reported to have potent antioxidant activity[31] thus suggesting its role in epilepsy.

B.monierri (Brahmi) is indicated in Ayurveda for treatment of epilepsy.[32] Crude plant extract of *B.monieri* or bacosides have shown anticonvulsive action. In a study by Kaushik D. et al.

ethanolic extract of *B.monierri* exhibited anticonvulsant activity in PTZ, MES and strychnine -induced convulsion in rats, hypoxic stress -induced convulsions in mice and lithium –pilocarpine - induced status epilepticus. It significantly increased the latency of onset of seizure in all models and in MES, it significantly reduced the duration of THLE with a mechanism of action similar to that of benzodiazepines (GABA agonist).[32] Khan et al. reported the neuroprotective role of *Brahmi* (methanolic extract) in hippocampus of temporal lobe of epileptic rats. *Brahmi* exerted neuroprotective effect by reversing the alterations in glutamate receptor binding and NMDA R1 gene expression that occur during epilepsy, resulting in reduced glutamate mediated excitotoxity in the over-stimulated hippocampal neurons.[33] Although *Bacopa* has been indicated as a remedy for epilepsy in Ayurvedic medicine, research in animals shows its anticonvulsant activity only at high doses over extended period of time.[34]

Oxidative stress is considered to be one of the contributing factor in epilepsy.[25] Leaves of *S.grandiflora* have been reported to have potent antioxidant activity.[31] All the plant material i.e. Vacha, Agasti, Brahmi used in *UGK* are shown to possess antioxidant activity .[28,31,25]

Though the exact mechanism of *UGK* is not clear, it appears to produce antiepileptic effect mainly through GABAergic[28,31,32] mechanisms which is developed after longer treatment i.e 8 days of administration of *UGK* (Figure 1 and 2). Antioxidant action of herbs present in *UGK* would impart additional antiepileptic activity along with neuroprotection.

UGK is a combination of minerals and herbs processed in traditionally validated methods. The probable action of this formulations could be by improving the therapeutic properties of each other with the increase in bioavailability of the formulation. Thus treatments with such polyherbal formulations could also be used as an adjuvant therapy for epilepsy. [35]

Conclusion

UGK appears to have significant antiepileptic activity after prolonged administration. The minerals and herbs together probably balance the excitatory and inhibitory neurotransmitters in CNS, the main action being GABAergic action and additional antioxidant activity of herbs. The combination of mineral with herbs seems rational. This study also supports the claim in ayurveda, of *UGK* being useful in treatment of epilepsy. However research is still needed to clarify the development of antiepileptic activity only after prolonged use of UGK. With wide spectrum of action this drug may be useful addition to antiepileptic agents which probably may be effective in all types of seizures. Further elucidation action in various animal models such as kindling and drug-drug interaction would open a new avenue in herbal biotechnology. Further research is in progress to evaluate the same.

Acknowledgement

Authors are thankful to Bharati Vidyapeeth Deemed University Pune, for providing us the required lab facility to carry out this work.

References

[1]. Fisher RS, Van Emde Boas W, Blume W, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 2005;46(4):470-2.

[2]. Giuliano Avanzini and L. Prilipko., Epilepsy Care in the World WHO 2005. Epilepsy Atlas, 2005

[3]. Werner F-M, Coveñas R. Classical neurotransmitters and neuropeptides involved in generalized epilepsy: a focus on antiepileptic drugs. Curr Med Chem 2011;18: 4933–48.

[4]. Brian S. Meldrum and Michael A. Rogawski. Molecular Targets for Antiepileptic Drug Development: *Neurotherapeutics*. 2007 January ; 4(1): 18–61.

[5]. Czuczwar SJ, Patsalos PN. The new generation of GABA enhancers. *CNS Drugs* 2001, *15*,339-350

[6]. Deckers CLP, Czuczwar SJ, Hekster YA, Keyser A, Kubova H, Meinardi H, Patsalos P, Renier WO, van Rijn CM. Selection of antiepileptic drug polytherapy based on mechanism of action: the evidence reviewed. *Epilepsia* 2000, *41*, 1364-1374

[7]. Sobieszek G, Borowicz KK, Kimber-Trojnar Z, Malek R, Piskorska B, Czuczwar SJ, Zonisamide. A new antiepileptic drug. *Pol. J. Pharmacol.* 2003, *55*, 683-689.

[8]. Löscher W. Current status and future directions in the pharmacotherapy of epilepsy. Trends Pharmacol. Sci.,2002, 23, 113–118.

[9]. Amos S, Kolawole E, Akah P, Wambebe C, Gamaniel K. Behavioral effects of the aqueous extract of Guiera senegalensis in mice and rats. Phytomedicine.2001;8:356-361.

[10]. Vd Pandit editor, Rasayogsagar, Haripranpannaji, Vol 1, Chowkhamba Krishnadas Academy, Varanasi, pp 177, verse 1666; 2004

[11]. Vd. Datto.Borkar, *RasaChandanshu* - *UnmadChikitsa* -13 Chapters. Publication-Ramesh Vitthal Raghuvansha, Shri gajanan book depo, Kabutar Khana, Dadar-Mumbai-28.

[12]. OECD, 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000

[13]. Ghosh M N. Fundamental of experimental Pharmacology; 3rd Ed.; Hilton and company Kolkatta, 2005. Chapter 30, page no.192-193.

[14]. Walker MC, Fisher A. (2004) Mechanisms of antiepileptic drug action. In Shorvon, S.D.,Perucca, E., Fish, D.R. and Dodson, W.E. (eds), The Treatment of Epilepsy . Blackwell: Oxford . 2004.

[15]. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Anti-epileptic drug development. II. Anticonvulsant drug screening. Epilepsia 1978;19:404–428

[16]. Sayyah M, Mandgary A, Kamalinejad M. Evaluation of the anticonvulsant activity of the seed acetone extract of *Ferula gummosa* Boiss against seizures induced by pentylenetetrazole and electroconvulsive shock in mice. J Ethanopharmacol 2002; 82(2-3): 105-109

[17]. Kaushik D, Kumar A, Kaushik P, Rana AC. Anticonvulsant activity of alcoholic extract of bark of Pinus roxburghii Sarg. J Chin Integr Med / Zhong Xi Yi Jie He Xue Bao. 2012; 10(9): 1056-1060.

[18]. Susanta Kumar Rout,1 Durga Madhab Kar. A review on antiepileptic agents, current research and Future prospectus on conventional and traditional drugs. *International Journal of Pharmaceutical Sciences Review and Research.* July –August 2010 , Volume 3, Issue 2,19-23

[19]. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 2006, 36:609–662.

[20]. Bandari Srinivasulu, Bhadra Dev H C. Murthy. Sodhana of gandhaka (sulphur) with godugdha (cow's milk), gogrutha (cow 's ghee): a chemical analysis]

[21]. Sawant Ranjeet, Bhoyar Manish. Pharmaceutics & Therapeutics of Kajjali (Black Sulphide of Mercury) - A review Asian Journal of Pharmaceutical Research and Development Vol.1 (3) May– June 2013: 92-97

[22]. Naveen Kodlady MS, Doddamani Y, Vishwanath BJ. Patgiri. Sedative Hypnotic Activity of Manashila(Realgar) – An Experimental Evaluation. Ancient Science of Life. Vol 30, No 3(2011), pg 78-83

[23]. Vishal Shah ND, Shah N D, Shinde SS. Solanaceae: Historical Aspect. International journal of pharmaceutical research And bio-science 2012: Volume1 (3):90-95

[24]. Hanan A. Al-Ashaal, Mona E. Aboutabl, Yousreya A. Maklad, Ahmed A. El-Beih. Tropane alkaloids of *Atropa belladonna* L.: *in vitro* production and pharmacological profile. Egyptian Pharmaceutical Journal 2013, 12:130–135

[25]. Wudayagiri Rajendra, Paturu Suseela, Gunduluru Swathi. Anticonvulsant Effect Of Different Extracts Of Bacopa Monnieri On Cholinergic Metabolism During Pentylenetetrazole -Induced Epilepsy. International Journal of Pharmacy and Biological Sciences 2013 Volume (3): 194-200

[26]. Sharma JD, Dandiya PC, Baxter RM and Kandl SI (1961). Nature: 192: 299

[27]. Jing-Kun Miao, Qi-Xiong Chen, Xiao-Mei Wu, Chun Li and Xiao-Ping Zhang, 2012. Antiepileptic Properties of Alpha-asarone from Acori Graminei Rhizoma in Mice and Rats Seizure Models. *International Journal of Pharmacology, 8: 567-571.*

[28]. Gopalakrishna HN, Sudhakar Pemminati, Shilin Giri, Ashok K Shenoy, GKS. Holla, Vinod Nair, Alwar MC, Sheethal D.Ullal. Effect Of *Acorus Calamus* On Electrical And Chemical Induced Seizures In Mice *International Journal of Applied Biology and Pharmaceutical Technology* Volume: I: Issue-2: Aug-Oct -2010

[29]. Hazra R, Ray K, Guha D. Inhibitory role of Acorus calamus in ferric chloride-induced epileptogenesis in rat. Hum Exp Toxicol. 2008; 26(12):947-53

[30]. Kasture VS, Deshmukh VK, Chopde CT. Anxiolytic and anticonvulsive activity of Sesbania grandiflora leaves in experimental animals. Phytother Res. 2002 Aug;16(5):455-60

[31]. Doddola S, Pasupulati H, Koganti B, Prasad KV. Evaluation of Sesbania grandiflora for antiurolithiatic and antioxidant properties .J Nat Med. 2008;62(3):300-7

[32]. Ali Esmail Al-Snafi. The pharmacology of Bacopa monniera. A review . International Journal of Pharma Sciences and Research. Dec 2013 Vol 4 No 12 ;154-159

[33]. Khan R, Krishnakumar A, Paulose CS. Decreased glutamate receptor binding and NMDA R1 gene expression in hippocampus of pilocarpine-induced epileptic rats: Neuroprotective role of Bacopa monnieri extract. Epilepsy and Behav 2008;12:54-60

[34]. Ganguly DK, Malhotra CL. Some behavioural effects of an active fraction from Herpestis monniera , Linn. (Brahmi). Ind J Med Res 1967;55:473-482.

[35]. Saxena VS, Nadkarni VV. Nonpharmacological treatment of epilepsy. Ann Indian Acad Neurol. 2011;14:148e152