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Evaluation of Anticonvulsant Activity of Synthetic and Herbal Drug Combination.

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ABSTRACT

Epilepsy is collectively designated for a group of chronic central nervous system disorders characterized by spontaneous occurrence of seizures generally associated with the loss of consciousness and body movements (convulsions). Anticonvulsant drugs are used to control the convulsions by inhibiting the discharge and then producing hypnosis. *Bacopa monniera* (BM), a traditional Ayurvedic medicine, used for centuries as memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent. In present study Synthetic and herbal drug combination was used to treat epilepsy. Antiepileptic drugs Phenytoin (25 mg/kg) and Diazepam (4 mg/kg) were used as standard synthetic drug, for Maximal Electroshock convulsion model and Strychnine induced convulsion model. *Bacopa monniera* (20 mg/kg) was taken as standard herbal drug which was used in combination with Standard antiepileptic drugs. In combination herbal drug was constant and synthetic drug dose was gradually reduced as 100%, 75% and 50%. In, MES induced convulsion combination shown complete protection of animals and reduces the extensor and stupor phases. Strychnine induced convulsion, the combination increases the onset of seizure but fail to protect the animals completely. The combination study suggests that, this could be due to changes neurotransmitter levels in brain. However, further research is necessary to determine the components involved and their mechanism of action in bringing about the desirable pharmacological effects.

Keywords: Convulsion, seizures, Anticonvulsant, herbal treatment, *Bacopa monniera.*

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INTRODUCTION

Epilepsy is one of the oldest neurological conditions known to humankind. The term ‘‘epilepsy’’ is derived from Greek word ‘‘epilambanein’’, which means ‘‘to seize upon’’ or ‘‘to attack’’[1]. Epilepsy, a disorder characterized by recurrent seizures[2]. A seizure is defined as an abnormal high frequency discharge of impulses by the nerve cells of the brain, resulting in a temporary disturbance of motor, sensory or mental function[3]. Epileptic seizures are a complex multiscale phenomenon that is characterized by synchronized hyperexcitationof neurons in neuronal networks [4].The abnormal amplification, synchronisation of neuronal firing that leads to seizure episodes and the ability of sub-groups of neurons to generate *intrinsic* burst-firing patterns is likely to involve interaction of GABAergic inhibitory mechanisms and glutamatergic excitatory mechanisms, particularly those involving NMDA receptor types[5].

Epidemiology

Epilepsy is a major and mostly preventable neurological disorder with an estimate of 69 million people worldwide. Recent meta-analyses showed a high estimate in developing countries; the median prevalence of lifetime epilepsy (LTE) was 15.4/1000 (4.8—49.6/1000) and 10.3/1000 (2.8—37.7/1000) respectively[6].

A recent meta-analysis of published and unpublished studies puts the overall prevalence rate of epilepsy in India at 5.59 per 1,000 population, with no statistically different rates between men and women or urban and rural residence. The worldwide prevalence of active epilepsy is between4 and 10 per thousand population[7].

India is home to about 10 million people with epilepsy (prevalence of about 1%)[8]**.**Recent community-based surveys have shown that epidemiological indices of epilepsy in India are comparable to those from developed countries, with a prevalence rate of ~5 per 1000 and incidence rate of ~50 per 100,000. Due to the shortage of neurologists and physicians in rural India, large numbers of epilepsy patient either do not receive therapy at all or tend to receive polypharmacy in irrational formats [9].

Mechanism of Epilepsy

Cerebrocortical dysfunction is the primary cause of epileptogenesis. Knowledge of normal cerebral cortex anatomy and cytoarchitecture is critical to an understanding of epilepsy. Normal CNS function relies on the initiation and transmission of excitatory impulses from one region to another. The majority of neurons in brain is excitatory and utilize glutamate as their excitatory neurotransmitter. In cerebral cortex the major glutaminergic neurons are pyramidal neurons, named for the roughly pyramidal shape of their cell bodies or somata .Within the six cerebrocortical laminae, most pyramidal neurons reside m laminae II/III and V/VI. The dendritic processes and somata of pyramidal neurons receive and integrate incoming or afferent signals. If the neuron is depolarized to a sufficient level by excitatory afferent inputs, an action potential is generated in the region of the axon hillock and propagates down the primary axon and its collaterals. Layer II/III pyramidal neurons send axonal projections to other cortical areas and to lamina V/VI pyramidal neurons (feedforward excitation). Pyramidal neurons in laminae V/VI project to subcortical areas. Normally, cortical pyramidal neurons are excited by thalamocortical afferents (afferent fibers originating in thalamus and projecting to cortex) or afferents originating in other cortical areas. The major pathway of excitation is an indirect one in which glutamatergic spiny stellate interneurons in lamina IV receive the afferent information and, in turn, excite neighboring pyramidal neurons m laminae II/III and V/VI. Spiny steflate neurons may also excite inhibitory interneurons(smooth or aspiny stellate neurons) which will, in turn, inhibit neighboring neurons (feedforward inhibition).Many inhibitory neurons also send collateral axons to more distant pyramidal neurons in adjacent cortical zones. This lateral inhibition results in the formation of an inexcitable area outside the primary afferent target zone called the "inhibitory surround" (Another form of inhibition is "feedback inhibition." Activated pyramidal neurons excite inhibitory interneurons which then feedback to inhibit the pyramidal neurons that had caused them to be activated. Under normal circumstances, all of these local and regional inhibitory circuits modulate activity within cerebral cortex and prevent abnormal spread of excitatory activity[10].

Neurotransmitters involved in epilepsy

GlutamateL-Glutamate is the most widespread amino acid in the brain and serves a number of functions in the CNS For example, this dicarboxylic amino acid is a precursor for the inhibitory amino acid neurotransmitter GABA, for the Krebs cycle intermediate a-ketoglutaratean for the amino acid glutamine. Glutamate also acts as a detoxification agent for ammonia products in the brain. In addition to the many metabolic functions of glutamate, the most significant role of glutamate in the brain is its function as the primary excitatory neurotransmitter.

Gama Amino butyric Acid (GABA) Deficiencies in the potent and widespread inhibitory neurotransmitter system operated by ɣ-aminobutyrate (GABA) have long been featured in putative aetiologies of epilepsy, and this subject has been extensively reviewed by others. Essentially, a selective loss of inhibitory GABA interneuron is conceived as a possible root cause of the disease. In recent years detailed re-examination of GABA levels in human temporal lobe tissue removed during surgery for epilepsy and has led to reports of clear and significant losses of GABA content which confirm and consolidate earlier reports, However losses of GABA content could result indirectly from dished levels of glutamate, the immediate biosynthetic precursor. Losses of benzodiazepine receptors in human focal tissue have also been recently reported.

This inhibitory amino acid is also featured in the first rational chemotherapies of epilepsy. Raising brain GABA levels appears to have an anticonvulsant action probably due to a rise in extracellular as well as intracellular levels Valproate (Epilim, Depakene) blocks GABA break down via the GABA shunt pathway and raises brain GABA levels 1.5 fold. It may also exert an anticonvulsant action by depressing the levels of the excitatory amino acid aspartate Another inhibitor of GABA breakdown, ɣ-vinyl-GABA, can raise brain GABA levels 5 to 7-fold, and is an effective anticonvulsant in a range of animal models. It is now proving to be effective clinically too[11].

In the last three decades, traditional systems of medicine have become a topic of global interest and importance. Current estimates suggest that, in many developing countries of the world, a large proportion of the people still rely heavily on traditional healers and medicinal plants for their daily primary health-care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity among the people for historical, cultural and economic reasons. In South Africa, plants have been used traditionally for medicinal purposes for many centuries. medicinal plants have been used for millennia in the effective treatment and/or management of various human and veterinary ailments [12].

*Bacopamonnieri*is a glabrous, succulent, creeping herb; stems are 10-30 cm long, rooting at the nodes with numerous ascending branches, leaves are round and ovate 6-10 m.m., flowers are light purple.[13]. it is distributed in tropics and subtropics of the world: in Sri Lanka, India, Nepal, China, Taiwan, Vietnam and Pakistan. It is also found in Florida, Hawaii and southern states ofUSA and the Mediterranean Basin.In India it is found in Andaman, Andhra Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Kerala,Karnataka, Manipur, Orissa, Punjab, Rajasthan, Tamil Nadu and West Bengal[14].Bacopa contains Alkaloids such as Hydrocotyline, Brahmine and Herpestine. Glycoside such as Asiaticoside andThanakunicide. Flavonoids such as Apigenin and Luteonin. Saponins such as D-mannitol, Acid A, Monnierin [C51H82O213H2O] Bacoside A [C41H68O134H2O] and Bacoside B [C41H68O135H2O]. Additional Phytochemicals such as Betulinic acid, Wogonin, Oroxindin, Betulic acid, Stigmastarol, beta-sitosterol, as well as numerous Bacosides and Bacopasaponins, and amino acids like alpha alanine, Aspartic acid, Glutamic acid, and Serine, and its esters, Heptacosane, Octacosane, Nonacosane, Triacontane, Hentriacontane, Dotriacontane, Nicotine, 3-formyl-4-hydroxy-2H-pyran (C6H6O3), and its 7- glucoside. Brahamoside, Brahminoside, Brahmic acid, Isobrahmic acid, Vallerine, pectic acid, fatty acids, tannin, volatile oil, ascorbic acid, thanakunic acid and asiatic acid . jujubacogenin and pseudojujubacogenin . In a thorough review of the chemical composition of Brahmi, the first constituent identified was an alkaloid brahmine. Saponins are considered to be the major active constituents of the plant. Saponins are glycosides, a sugar unit attached to an aglycone portion (the sapogenin). The sapogenin portion describes the type of saponin — either steroidal (4- ringed structure), or triterpenoid (5-ringed structure)[15]. Traditional uses are Adaptogenic, anticonvulsant, hepatoprotective, antidepressant, antianxiety, antispasmodic, sedative, potent nervine tonic, astringent, diuretic, hystriaetc[16].

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MATERIALS AND METHODS

Plant Material

The extract of *Bacopamonniera*in capsule was procured form (Himalaya Herbal Health Care, Batch no.-222002356)

Instrument and Equipments

Electroconvulsiometer, Electronic Balance, Oral Gavage.

Chemicals and Drugs

Gum Acacia (Sigma Aldrich, USA), Strychnine (Sigma Aldrich, USA), Phenytoin (Organic Chemicals Private Limited, Thane, Maharastra, India), Biopose (Diazepam Injection, Biochem Pharmaceutical Industries Limited).

Pharmacological Activities

Animals and Source

Swiss Albino mice weighing 18-30g of either sex were used in this study after approval of the institutional Animal Ethics Committee. (No-UIP/CPCSESA/J/2013/11)Mice used for the study were obtained from the animal house of BabuBanarasi Das National Institute of Technology and Management, Lucknow, U.P, India.Mice were housed in clean cages. The bedding material of the cages was changed every day. The animals were maintained under natural day and night cycle. They were kept on standard pallet diet and water ad libitum. They were initially acclimatized to the laboratory environment for seven days prior to their use.

Maximal Electroshock (MES) Induced Convulsions

The anticonvulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions[17].The animals were weighed and selected for the experiment depending on the weight. Mice of either sex were used. The mice were then divided into 6 groups of 6 mice each. Group 1 received vehicle; group 2 received 25mg/kg b.wt. of Phenytoin 60 minutes prior to the convulsion induction. Group 3 received standard 2 drug; group 4 received combination of Standard 1 and standard 2; group 5 received of standard 1 and standard 2; and group 6 received of standard 1 and standard 2,60 minutes before to the convulsion induction. The Maximal electroshock of 50 mA current for 0.2 Sec was administered through corneal electrodes to induce convulsions in the control and drug treated animals. All the animals were treated for 14 days. The ability of the drugs to prevent or delay the onset of hind limb extension was taken as an indication of anticonvulsant activity.

Strychnine Induced Convulsions

Strychnine and related alkaloids such as brucine and thebaine induce generalized convulsions after systemic administration. The anti-convulsant property of the drug in this model was assessed by its ability to protect against strychnine induced convulsions[18].The animals were weighed and selected for the experiment depending on the weight. Mice of either sex were used. The mice were then divided into 6 groups of 6 mice each. Group 1 received vehicle; group 2 received 4 mg/kg b.wt. of standard 1 (diazepam); group 3 received standard 2; group 4 received combination of Standard 1 and standard 2; group 5 received of standard 1 and standard 2; and group 6 received of standard 1 and standard 2 respectively; 60 minutes prior to the strychnine (2.5. mg/kg b.wt.) administration. Strychnine produced powerful tonic convulsions of the body and limbs. All the animals were treated for 14 days. The latency of convulsions and the % mortality was assessed for each animal for 30 minutes.

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OBSERVATION AND RESULTS

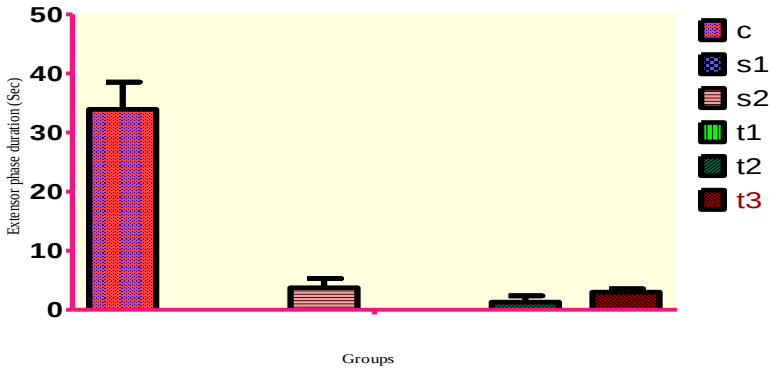
Maximal Electroshock Model

The results are shown in Table-1 PHT (25 mg/kg b.wt.) and *Bacopamonniera* (20 mg/kg b.wt.) ($p < 0.001$) shown complete abolition of extensor phase in acute and chronic studies of MES induced convulsions.

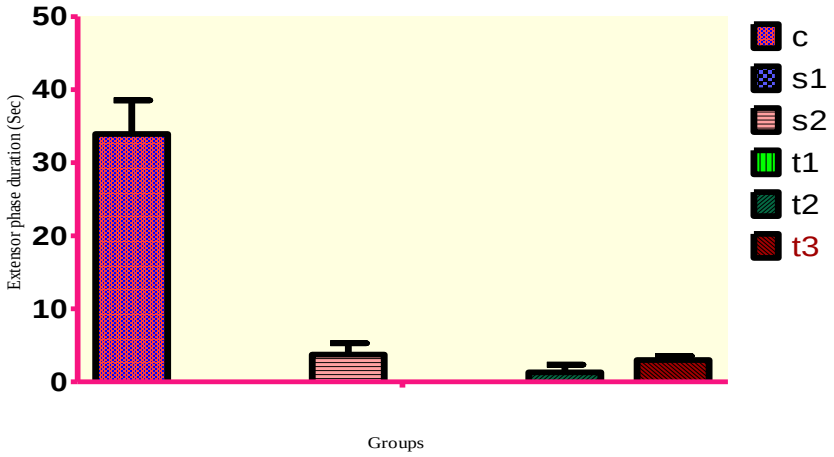
Table 1: Effect of Phenytoin and *Bacopa monniera* extract in Maximal Electroshock Method in Acute and Chronic Studies in Mice

Sr. no.	Groups (n= 6)	Extensor Phase			
		Acute Study	Chronic Study	% Protection	
				Acute Study	Chronic Study
1	Control	8.88 ± 0.27	33.93 ± 0.77	0	0
2	Std 1	0.00***	0.00***	100	100
3	Std 2	1.58 ± 0.04***	3.73 ± 0.26***	100	75
4	Test 1	0.00***	0.00***	100	100
5	Test 2	0.92 ± 0.12***	1.30 ± 0.18***	100	100
6	Test 3	1.53 ± 0.03***	3.0 ± 0.09***	100	75

Statistical significance test was done by ANOVA followed by Tukey's Test. Values are Mean ± S.E.M. of 6 Animals per group, Significance $P < 0.001$ vs. control group.



Effect of Phenytoin, *Bacopa monniera* extractand their combination on extensor phase in acute studyon MES induced seizures in mice.



Effect of Phenytoin, *Bacopa monniera extract* and their combination on extensor phase in chronic studyon MES induced seizures in mice.

Strychnine Induced Convulsion

The Results are shown in Table 2, Diazepam and B.M. significantly prolong the onset of jerk when compared with control in both acute and chronic studies. The onset of jerk in animals treated with Phenytoin at different doses increases in combination with the extract of *Bacopa monniera* (20mg/kg) significantly prolonged the onset of jerk. Diazepam, B.M. and their combination also show significant reduction in the duration of convulsion when compared to the control in acute ($p< 0.001$, $p<.01$) and chronic ($p<.05$, $p<.01$) studies. Onset of seizure also prolonged by the Diazepam, B.M. and their combinations in acute and chronic studies significantly ($p<.05$, $p<.01$ and $p<.01$).

Table2:Effect of Diazepam, *Bacopa monniera* extract and their combination in Strychnine induced convulsion in Acute and Chronic Studies in Mice.

Sr. no	Groups	Onset of jerk		Duration of jerk		Onset of seizure	
		Acute	Chronic	Acute	Chronic	Acute	Chronic
1	Control	294.40±12.58	362±23.17	143.33±8.48	295.83±31.69	430.40±18.53	681±11.55
2	Std 1	540±19.29**	754±29.48***	60.00±3.65**	101.67±14.47*	592±20.49**	884±33.78
3	Std 2	488±18.94*	632±13.25**	50.83±6.06***	86.67±5.84*	527±18.82*	728±10.17
4	Test 1	536.67±18.55**	720±22.48***	56.67±4.79**	103.33±14.36	586±21.40**	832±12.61
5	Test2	478±15.56*	642±19.31	49.17±6.11***	71.67±6.70**	529±20.06*	708±22.22
6	Test 3	476±11.64*	600±14.72	45.83±2.71***	70±0.00**	519±11.03*	664±21.65*

Statistical significance test was done by ANOVA followed by Tukey's Test. Values are Mean ± S.E.M. of 6 Animals per group, Significance $p<0.001$, $p<0.01$, $p< 0.1$.

DISCUSSION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterized by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons[19].The maximum electroshock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. Antiepileptic drugs may produce their effects by the following mechanism normalization of seizure foci, prevention of the origin of seizures from the foci, prevention of post-titanic potential, elevation of excitatory synaptic threshold, potentiation of pre or post synaptic inhibition, prolongation of the refractive period. Phenytoin inhibits Na^+ channels, but diazepam act through GABA (Gaba amino butyric acid) producing pharmacological action. Phenytoin does not affect chemically induced seizures but prevent tonic convulsions produced by MES. Diazepam prevents chemically induced seizures and it is drug of choice of status epilepticus, but is having sedative action and development of tolerance[20].

Bacopamonniera and bacoside-A treatment were found to enhance the GABA receptor binding[21].Although there are various reports of the anticonvulsant and neuroprotective properties of *B. monnieri*, little effort has been extended to understand their pharmacological action on the glutamate receptors in hippocampus, the site most afflicted in epilepsy. Glutamate dehydrogenase activity is high in hippocampus in association with pilocarpine-induced epilepsy, and was brought down to near-control levels after treatment with *B. monniera* extract. Increased glutamate dehydrogenase and decreased glutamate decarboxylase are indicative of the accumulation of glutamate. Glutamate is one of the chief excitatory amino acids that mediate excitotoxic neuronal degeneration. Treatment with *B. monniera* extract reduced the increase in glutamate dehydrogenase activity to near-control levels. Hence, it is suggested that *B. monnieri* has a definite role in decreasing glutamate excitotoxicity[22].

The present study demonstrates a dose dependent increase in the seizure threshold, suggesting that the extract might possess anticonvulsant property against the MES and Strychnine model. By and large, the traditional system of medicine is slow-acting as compared to the modern synthetic drugs because they are administered as crude preparations. Keeping this in view, the anticonvulsant efficacy of *B.monnieri*was examined following daily administration for 14 days. The results of the chronic administration also clearly demonstrated that this extract increased the seizure threshold. However, 14 days treatment had no advantage over single dose treatment.

From our study clearly shown that *B.monnieri* has an influence on the excitatory and inhibitory neurotransmission, of special interest being the increase in the gamma amino butyric acid (GABA) levels In this study we have demonstrated the possible synergistic interaction of *B.monnieri*extract and Standard

antiepileptic drugs and its beneficial effect in combinations. The increased percentage of complete protection in combination treatment demonstrated the enhancement of the response of Standard antiepileptic drugs by*B.monnieri*. Moreover, many investigators have suggested that the experimental protective index could express better the clinical utility between the relationship of undesired and the desired drug effect.

The findings indicate a pharmacodynamic interaction of the combination drug regime but do not rule out the contribution of pharmacokinetic interaction, since there was no any type of toxicity found during the studies. The combination study underscores the significance of the synergistic effect of *Bacopa monniera* in combination with standard antiepileptic drugs (Phenytoin and Diazepam) where the dose of the latter is considerably reduced, the combination study suggests that, this could be due to changes in brain neurotransmitter levels[23]. However, further research is necessary to determine the components involved and their mechanism of action in bringing about the desirable pharmacological effects and explore the full potential of *Bacopa monniera* in epilepsy and correcting antiepileptic drug induced side effects on Central nervous system.

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