ISSN2277-2685
IJESR/March. 2022/ Vol-12/Issu1/1-08
B.Santosh Kumar *et.al.*,/International Journal of Engineering & Science Research

Anticonvulsant potential of commonly practiced formulations of *Brahmi* (*Bacopa monnieri* Linn.) inWistar rats

B.Santosh Kumar[1], K. Manjulatha[2], R.Krishna Priya[3]
Associate. Professor[1], Asst.Professor[2,3]
bonisantosh45@gmail.com
Sri Sivani College of Pharmacy

A B S T R A C T

Objective: Brahmi (*Bacopa monnieri* Linn) is an important herb in Ayurved, reported to have a wide range of medicinal properties. In clinical practice it is usually prescribed in its various dosage forms. The most common of those are *Brahmi Ghrita* (BG) and *Saraswatarishta* (SW). Use of *Brahmi* as anti-convulsion drug is well documented in scientific literature however; no data is available on the effect of its commonly practiced dosage forms. Hence, the study was carried out to evaluate anti-convulsion potential of BG and SW.

Method: The anticonvulsant activity of BG and SW was studied against seizures induced by Maximal Electroshock (MES) in rats and phenytoin (25 mg/kg Intra Peritoneal) was used asstandard. Different phases of convulsions (Hind limb extension, jerking, grooming, tail straub and recovery) were recorded as index of convulsion. The brain tissue was dissected out for biochemical analysis.

Result: Treatment of rats with SW and BG in Maximal Electroshock (MES) induced con- vulsions showed statistically significant potential as compared to control groups ($P \leq 0.01$ or $P \leq 0.001$). SW or BG showed significant improvement in all the phases of convulsion except grooming response. Brain tissues of test animals evaluated for malondialdehyde (MDA) levels showed the higher levels in phenytoin group than in BG and SW treated groupsuggesting protection of brain tissue from oxidative damage.

Conclusion: The results indicated SW and BG to be effective in promoting restorative and neuroprotective action in convulsions thus suggesting a further scope of evaluation of these formulations as an adjuvant treatment for convulsions

Introduction

Medhya drugs are the best gifts of traditional Ayurvedic system to mankind, which are commonly used for maintenance as well as treatment for a range of neurocognitive disorders. Many herbal, mineral and animal drugs are being practiced with the potential to be used in such conditions.[1] Single herbs and polyherbal formulations like *Brahmi* (*Bacopa monnieri* Linn), *Vacha* (*Acorus calamus* L.), *Shatavari* (*Asparagus race- mosus*), *Brahmirasayan* etc. mainly categorized in this special-ized group of *Medhya* drugs and have a long history of use in their myriad effects on the Central Nervous Systems.[2]

Of all these, *Brahmi* is one of the most commonly used herbs, the neurocognitive effects of which are well estab-lished.[3] The herb although very commonly practiced by Ay- urvedic fraternity, it is mainly used in the form of its polyherbal formulations like *Saraswatarishta* (SW) and *Brahmi Ghrita* (BG), *Saraswat Choorna* etc. Other drugs associated withthe herb and dosage form prepared is anticipated to boost the potential of herb and to reduce therapeutic dose. Most of the studies are found on evaluating neurocognitive benefits of these formulations.[4e7] In the traditional practice however formulations are also being used for their promising action on epileptic conditions to prevent the attacks and reduce after effects with reference to cognitive deficits.[8] However, very fewstudies can be found in evaluating these effects of the formulations.

"Epilepsy" is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and imbalance in brain electrical activity[9] which is commonly correlated to "*Apasmara*" or "*Apasmriti*" (loss of consciousness or memory) in Ayurved. It is the second most unrelieved common neurological disorder[10] fundamentally involving different neurological conditions/disturbances and symptoms with varying disease etiology in different people.[11,12] A known characteristic feature of epilepsy is seizures (periodic neuronal discharge), which is becoming important medical problem and needs urgent remedy.

Currently a number of Antiepileptic drugs (AEDs) are in practice with some beneficial effects, but none of these drugs can completely control seizures. Along with this, a number ofside effects are eventually increasing the cost for epilepsy careand drug induced morbidity.[13,14] Thus it becomes imperative to search for a safer and potential alternative to the existing treatment from traditional medicinal systems. This study aims to evaluate the anti-convulsion potential of commonly used formulations BG and SW with well-known antiepileptic drug Phenytoin as standard by using Maximal Electroshock (MES) induced convulsions. ingredients for the formulations were collected from a local *Ayurvedic* vendor and identified by *Ayurvedic* practitioner. Both the

formulations *Brahmi Ghrita (*BG) *and Saraswatarishta* (SW) were prepared and standardized in accordance with Ayurve- dic Formulary of India.[15,16]

Chemicals

Phenytoin and Tetramethoxypropane (TMP) was procured from Sigma Aldrich Co., St. Louis, USA used as positive control and standard respectively. All the other chemicals used for biochemical estimation like Potassium chloride (KCl), Thio- barbituric acid (TBA), Trichloroacetic acid (TCA), Hydrochloric acid (HCl) and Butylated hydroxytoluene (BHT) were of analytical grade, obtained from Qualigen fine chemicals Pvt. Ltd. Mumbai.

Experimental animals

Wistar (Albino) rats of either sex (140e200 g) were procured from National Toxicology Center, Pune. The animals were allowed to acclimatize for eight days. Housed and maintained in standard laboratory conditions fed with standard rat pellet diet and water *ad libitum*. The experiment was conducted with prior permission of Institutional Animal Ethical Committee (IAEC Ref. No. 884/ac/05/CPCSEA) and according to the Com-mittee for the Purpose of Control and Supervision on Experi- ments on Animals (CPCSEA) guidelines.

Experimental designs

Animals were divided into four groups (*n* ¼ 6); Group I served as control group and received only water and feed *ad libitum*, Group II received standard drug Phenytoin (25 mg/kg IP), Group III and Group IV received Brahmi Ghrita (BG) (0.9 ml/kg) and Saraswatarishta (SW) (0.9 ml/kg) orally for eight days respectively, at a fixed time in the morning. The dose was decided according to the therapeutic human dose of the for- mulations extrapolated to animals.[17]

Assessment of anticonvulsant activity by Maximal Electroshock (MES) induced convulsions

MES seizures were induced by Electro-convulsometer (Medi-craft Electro Medicals P. Ltd.) as described by Swinyard[18] (1985). Exactly 1 h after the drug administration, maximal electroshock seizures were elicited by the application of electric shock (60 Hz AC, 150 mA) for 0.2 s (s) using cornealelectrodes. This current intensity brought forth complete tonic extension of hind limbs in control rats. For recording

1. Materials and methods

Preparation of formulation

Brahmi (*B. monnieri*), the main ingredient of formulations was collected from natural habitat early in the morning. Voucher specimen was confirmed and deposited in the herbaria of Medicinal Plants Conservation Centre, Pune (MPCC 959). Other ting full view of the animal motor responses to seizure. Duration of various phases of epileptic attacks like jerking, grooming, tail straub, extension of hind limb and recovery were observed, recorded and compared with the control and phenytoin group. Animals were sacrificed by cervical dislo- cation and brain tissues were isolated immediately, washed with ice cold Phosphate Buffer Saline (PBS) and stored at
—80 °C until further use.

Table 1 e Effect of *Brahmi* formulations (BG and SW) on various phases of convulsions against control.

Groups	Hind limb extension (s)	Jerking (s)	Grooming (s)	Tail straub (s)	Recovery (s)
Control	1.226[a] 0.07	0.908[a] 0.02	0.693[a] 0.03	1.074[a] 0.04	2.619[a] 0.01
BG	0.622[b,c,d,***] 0.23	0.577[c,d,**] 0.07	0.656[a,**] 0.07	0.672[e,***] 0.05	2.221[c,d,***] 0.04
SW	0.658[b,c,d,***] 0.22	0.476[c,***] 0.07	0.654[a,**] 0.08	0.577[e,***] 0.07	2.228[d,***] 0.08
Phenytoin	0[e,***]	0.259[b,**] 0.06	0.310[b,**] 0.07	0.709[e,***] 0.02	2.124[e,***] 0.02
Bartlett Statistic (*P* Value)	6.113 (0.1063)	4.956 (0.2918)	5.508 (0.2391)	4.495 (0.3431)	14.718 (0.0053)

Values represent mean SEM (n ¼ 6).
BG is Brahmi Ghrita, SW is Saraswatarishta, SEM is the standard error of the mean, n ¼ 6 observations.
The figures followed by different superscript letters in a column are statistically different.
*** $P \leq 0.001$ as compared to control; ** $P \leq 0.01$.

Biochemical analysis: estimation of malondialdehyde(MDA) content (lipid peroxidation assay)

Estimation of lipid peroxidation in brain tissue was measured by using the method of Ohkawa et al 1979.[19] Brain homoge- nate was prepared in PBS (10%) and One ml of 0.15 M KCl wasadded to 0.5 ml of homogenate. It was incubated for 30 min at 37 °C (degree centigrade) and the reaction mixture was treated with 2 ml of TBA- TCA-HCl reagent, 0.2 ml of BHT and heated for 60 min at 80 °C. It was centrifuged for 10 min and super- natant was used. Spectrophotometrically (Biorad SmartSpec Plus) absorbance was measured at 532 nm and values were expressed in mM of MDA/gm of tissue. 1,1,3,3-Tetramethox- ypropane (TMP) was used as a standard.

2.5. Statistical analysis

The statistical analysis was done by using InStat (Trial Version3.06). The data values were log transformed before analysis.The data were analyzed for Kolmogorov and Smirnov's Gaussian distribution test and Bartlett statistics was applied to assess the differences between standard deviations of the populations from which the samples were drawn. The data were subjected to Dunnett's multiple comparison tests to compare the means of different groups and to calculate sta- tistical significance amongst the groups. Analysis of variance ANOVA was carried out in order to determine the intra andinter-group variations.

Brahmi (*B. monnieri*), a potent nootropic drug[3,22] is also studied for its anticonvulsant activity in albino rats, using various convulsive models.[6] In our study, two most commonly used dosage forms of this well-known drug; BG and SW were evaluated for their anti-convulsion activity against Phenytoin and different stages were recorded on 8th day of experiment on all four groups.

BG produced a more significant effect in phase of extension(0.622 0.23 s) and recovery (2.221 0.04 s) compared to control($P \leq 0.001$) (Table 1). Both the formulations showed decrease in extension time as compared to control ($P \leq 0.001$), which sig- nifies the formulation efficacy to prevent the spread of seizure in the central nervous system.[6,23] SW was found to be more effective in improving jerking and tail straub as compared to control ($P \leq 0.001$). BG and SW did not show statistically sig- nificant improvements in grooming when compared to phenytoin treated group ($P \geq 0.1$) but significant improvements were observed as compared to control ($P \leq 0.01$). Both the formulation significantly reduced duration and recovery time of MES induced convulsions in rat (18.3 0.2 s, 17.0 0.4 s, and 166.3 1.6 s, 169.3 3.3 s respectively) as compared to control(42.4 2.5 s, 415.8 1.2 s) (Table 2) (Fig. 1). Inhibition of MES induced convulsions predicts activity against tonic clonic sei- zures that needs to be explored further.

Maximum Electroshock induced seizure (MES) ratmodel

The MES induced epilepsy model has most frequently beenused to elucidate potential of antiepileptic drugs. Most of these compounds like phenytoin, sodium valproate, felba- mate are known to display the same ability to inactivate voltage dependent Na^{þ} channels in a use dependent fashion[6]or by blocking glutamatergic receptor. Inhibition of a major inhibitory neurotransmitter Gamma-Amino Butyric Acid(GABA) and enhancement of the action of glutamic acid in brain also have been shown to be the contributory factors in epilepsy.[20] Data from several studies have identified the use oftraditional herbal medicines for epilepsy using the same (MESinduced) models.[14,21] treated group was higher 138.82 0.094 (mM/g tissue) than inBG and SW treated group (93.60 0.636 and 48.82 0.456 mM/g tissue respectively) which was comparable to control group(50.16 0.016 mM/g tissue).

Table 2 e Anticonvulsant effect of *Brahmi* formulations (BG and SW) on Maximal Electroshock induced Seizures in albino Wistar rats.

Groups	Duration of convulsion (s)	Recovery time (s)
Control	42.4 2.5	415.8 1.2
Phenytoin (25 mg/kg i.p)	9.5 1	133.2 0.8
BG (0.9 ml/kg b.o)	18.3 0.2	166.3 1.6
SW (0.9 ml/kg b.o)	17.0 0.4	169.3 3.3

Values represent mean SEM (*n* ¼ 6).
BG is Brahmi Ghrita, SW is Saraswatarishta, SEM is the standard error of the mean, *n* ¼ 6 observations.

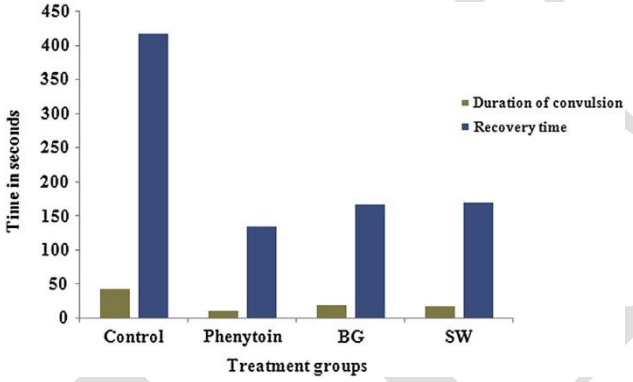

Fig. 1 e Anticonvulsant effect of *Brahmi* formulations on duration and recovery time of MES induced convulsions.

2. Discussion

Present study was set out to validate the traditional use of BG and SW for their protective and restorative potential in epi- lepsy. The *in vivo* and biochemical findings add to our under- standing of anti-convulsive potential of *Brahmi's* commonly used formulations (BG and SW). Earlier studies suggest that delayed latency of the seizures is probably by balancing level of both GABA and glutamic acid.[20] The formulations might have action in similar manner but probable mechanisms of action for these formulations need to be explored in detail.

Brahmi Ghrita is a polyherbal formulation contains base as *Ghrita* i.e. Cow's ghee[24] and acts as a beneficial therapeutic formulation by providing good absorption, assimilation and delivery to the target organs due to its lipophilic nature.[25,26] Whereas SW is a fermented hydroalcoholic dosage forms of *Brahmi* as a major ingredient having a wide therapeutic use. Both of the formulations although clinically evident to have a potential role in epilepsy, no study has scientifically docu- mented the efficacy. Our study has shown that BG and SW both have comparable potential in protecting the epilepticseizure intensity and fostering recovery.

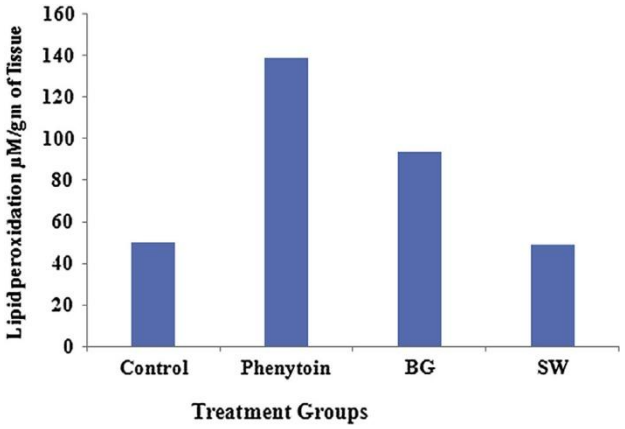

Fig. 2 e Effect of *Brahmi* formulations on level of brain lipidperoxidation.

Contemporary treatments for epilepsy have a major side effect of cognitive defect, which cannot be undermined as antiepileptic treatments generally continue over the years.[27,28] On the other hand, SW and BG have been proven to have a cognition enhancing effect. Thus on the grounds of their role in epilepsy and a major role in learning improve- ments, these formulations can emerge as a better and safer alternative to current treatments. However, a detailed evalu- ation of this aspect using preclinical and clinical studies is needed. As these drugs are a combination of many herbs and processed in traditionally validated methods, the probable role of these formulations could be by improving the thera- peutic properties of *Brahmi* alone with the increase in bioavailability of herbal.[29,30] Thus treatments with polyherbal formulations could also be used as an adjuvant therapy for epilepsy.[31]

Reactive oxygen species have been identified as the most crucial factor in neuronal damage because of rich PUFA con- centration in the brain tissue.[32,33] Increase in oxidative stress damages of the neurons, which are known to have a minimal regenerative capacity. In MES induced seizures the MDA levels, which represent oxidative stress in the brain suggested a significant damage in case of control rats. However, in the treatment control group of Phenytoin, the damage was much higher suggesting a potential damage of brain tissue by the treatment. In the BG and SW treated groups, these readings were significantly lower even from the control and phenytoin which again validates the traditional protective role of these formulations in epilepsy related tissue damage.

3. Conclusion

From the present study, we can conclude that both the for- mulations of *B. monnieri* i.e. *Brahmi Ghrita* and *Saraswatarishta* have promising anti-convulsive activity with better restor-ative effects. Phenytoin has been proven to be most significant as compared to herbal drugs in controlling convulsions but the oxidative damage by the drug can be controlled or side- lines by the use of concurrent Ayurvedic polyherbal treat- ments. This scientific evidence for the Ayurvedic therapies can make effective health care and patient friendly medica- tion for convulsions. Further elucidation of mechanism of action and drugedrug interaction would open a new avenue inherbal biotechnology.

Conflicts of interest

All authors have none to declare.

REFERENCES

A team including Aneesh TP, Hisham M, Sekhar MS, Madhu M, and Deepa TV developed this. Traditional Indian herbal remedies are seeing a decline in the international market. "International Journal of Green Pharmacy" 2009; 3: 184–190.

2. (Tyagi A, Delanty N.) Supplements, herbal medicines, and epilepsy. Journal of Epilepsy. 2003;44(2): 228–

235.

3. (Brahmi), an Ayurvedic nootropic, Bacopa mooniera Linn., and its neuropsycopharmacological effects, by Singh HK and Dhawan BN. Journal of Indian Pharmacology. 1997;29:359–365.

4. Anandasundaram ARBE, Akbar Mohammed, and Radha Shanmugasundaram Khritham K. Brahmi

Herbal Ayurvedic formulation for epilepsy management. Published in 1991 in the Journal of Ethnopharmacology, volume 33, pages 269–276.

5. Jain S, Tandon P. Indian literature on epilepsy and Ayurvedic medicine. Neurol Asia. 2004;9:57–58.

6-Darpan K, Tripathi A, Tripathi R, Ganachari M, Khan SA. Rats given Bacopa monniera showed no evidence of convulsive seizures. "Brazilian Journal of Pharmaceutical Sciences, 2009, 45: 643–649."

Saraswatarishta's impact on memory and learning was studied by Uma, Kavimani, and Raman (2007). The International Journal of Plant Pharmacology reported on this in 2010 with a page range of 15–19.

Ayurvedic formulas for the treatment of epilepsy (Kumar D, Kumar S, Murthy KH, Narasimha SS, 2008). The International Journal of Pharmaceutical Research, 2012, 3, 17–20.

9. The authors include Robert F., Walter V., Warrem B., and others. Proposed definitions of epilepsy and epileptic seizures by the International Bureau for Epilepsy and the International League against Epilepsy. Journal of Epilepsy. 2005;46:470–472.

Vitex agnus castus extract's antiepileptic effects on amygdala-induced seizures in male rats was studied by Saberi, Rezvanizadeh, and Bakhtiarian (2010). Journal of Neuroscience, 2008, 441, 193–196.

11. Berg AT, Levy SR, Testa FM, Shinnar S. Newly diagnosed epilepsy in children: interrater agreement and disagreement grounds for syndrome classification. "Epilepsia" (1999, 40:439–444).

12. Visweswari G, Siva Prasad K, Lokanatha V. Centella asiatica's antiepileptic effects on the magnesium (Mg) and calcium (Ca) ATPases in the brains of rats subjected to pentylenetetrazol-induced epilepsy. Journal of Indian Pharmacology. 2010;42:82–86.

13. The research was conducted by Sonavane GS, Palekar RC, Kasture VS, and Kasture SB. Myristica fragrans seeds have anticonvulsant and behavioral effects. Indian Journal of Pharmacology. 2002;34:332e338.

Article 14: Vyawahare NS, Khandelwal AR, Batra VR, and Nikam AP. Journal of Herbal Medicine and Toxicology, 2007;1(1):9–14. Herbal anticonvulsants.

$15. Ayurvedic Pharmacopoeia Committee, anonymous. The Indian Ayurvedic Compendium. India: Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homoeopathy; 2003:279–280.

New Delhi, India: Government of India, 2003.

16. The Ayurvedic Pharmacopoeia Committee, who want anonymity. The Indian Ayurvedic Compendium. The Indian System of Medicine and Homoeopathy was published in 2003 by the Government of India in New Delhi, India, from pages 97 to 99.