MULMINA™ REVERSES MEMORY IMPAIRMENT INDUCED BY GABAPENTIN IN PTZ EPILEPTIC MOUSE MODEL AND EXHIBITS SYNERGISTIC ANTIEPILEPTIC ACTIVITY

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ABSTRACT

Epilepsy is a neurological condition that causes unprovoked, recurrent seizures and memory impairment is a common side effect of epileptic treatment. The present study was conceptualized to evaluate the protective effect of the nootropic herbal drink Mulmina™ against memory impairment induced by Gabapentin in a pentylenetetrazole-induced epileptic mouse model. The antiepileptic and memory impairment activity were found to be significantly ($p<0.05$) higher in the gabapentin group, whereas the combination of gabapentin + Mulmina™ significantly ($p<0.05$) increased antiepileptic and decreased the memory impairment activity. Furthermore, Mulmina, alone exhibited synergistic antiepileptic and memory enhancement activity. Thus, combining herbal drugs/nootropics with anti-epileptic drugs provides synergistic activity while lowering the dose of synthetic drugs, which may cause more adverse effects in the human body. The results of this study show that gabapentin has memory impairment potential and that it can be corrected by co-administration of Mulmina™. However, future research is warranted to assess the underlying molecular mechanism of memory enhancing activity of Mulmina™ against gabapentin induced memory impairment.

Keywords: Gabapentin, Mulmina™, Temporal Lobe Epilepsy, Memory Impairment, Antioxidant

INTRODUCTION

Epilepsy is a chronic brain disorder causing abnormal movement of the body, unconsciousness and psychotic phenomena[1-2]. There are various forms of epilepsy that exhibit different signs and symptoms, some of which are in complex conditions and some of which are difficult to classify. Signs and symptoms of seizures include such as body jerking motions that are uncontrollable and uncoordinated, temporary distraction, loss of consciousness, a staring spell and symptoms such as fear and anxiety[3]. In people with epilepsy, co-morbid conditions are widespread and their existence provides valuable information for diagnosis, treatment, medical costs and quality of life. Nearly 50% of adult epileptic patients have at least one co-morbid medical disorder, which include medical, cognitive, psychiatric, alone, or in combination[4].

Lies there are two types of epilepsies namely; partial and generalized. Partial seizures are further classified as simple and complex, whereas atonic, myotonic, tonic-clonic and tonic are classifications of generalized seizure[5-6]. Partial seizure happens in one hemisphere while the epileptic condition is limited to the brain networks. Both the left and right hemispheres of the brain experience the generalised type of seizure[7-9]. Memory impairment (MI) is one of the major disadvantages of epilepsy-related to irregular functions of the brain's excitatory and inhibitory neurotransmitters that contribute to stress and eventually to memory collapse. Single-seizure manifestations are unlikely to cause permanent damage[10-12]. Temporal failure is the core area of the learning process and new memory mechanism is suppressed by damage to the temporal lobe, this is known as amnesia anterograde[13-14].

One of the main issues associated with epileptic patients is memory problems or memory deficiency. All of this is due to the neurotransmitters or neuromodulators

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Table I: Grouping of experimental animals, treatment and evaluation

Group	Treatment	Evaluation
Normal	0.5% Sodium CMC for 29-day oral use as vehicle	Durations of convulsion induced by PTZ method were noted on 1st, 7th, 14th, 21st and 28thday and MI activity was noted by RAM on 2nd, 8th, 15th, 22nd and 29th day.
Control	Vehicle was administered for 29 days and through PTZ convulsion was induced (45 mg kg^{-1}, i.p) method on 1st, 7th, 14th, 21st and 28th day	
Phenytoin+PTZ	Phenytoin (25 mg kg^{-1}) was given as suspension in vehicle (i.p) for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p)	
GBP (Normal Dose) + PTZ	GBP (200 mg kg^{-1}) was given as suspension in vehicle (i.p) for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p)	
GBP (Reduced Dose) +PTZ	GBP (100 mg kg^{-1}) was given as suspension in vehicle (i.p) for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p)	
GBP (Normal Dose) +Mulmina + PTZ	GBP (200 mg kg^{-1}) and Mulmina (80 mL kg^{-1}) was given as suspension with vehicle (i.p and P.O) at the interval of 2hr and 1hr for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p)	
GBP (Reduced Dose) +Mulmina + PTZ	GBP ½ dose (100 mg kg^{-1}) and Mulmina (80 mL kg^{-1}) were given as suspension in the vehicle (i.p and P.O) at the interval of 2hr and 1hr for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p).	
Mulmina + PTZ	Mulmina (80 mL kg^{-1}) was given orally for 29 days before inducing convulsions by PTZ (45 mg kg^{-1}, i.p).	
	n=6 per group	

involved in neuronal excitement. Abnormal functions between the brain's excitatory and inhibitory neurotransmitters result in tension and brain tiredness, which in turn causes a memory lapse. Cognitive impairments associated with seizures are by multiple factors likewise biological (underlying frequency, neuropathology, duration of seizure, etiology, structural cerebral damage and severity), psychosocial and treatment-related factors. Antiepileptic drugs can cause some of the adverse effects like memory impairment, psychomotor slowing, and reduced vigilance by suppressing functions of excitatory neurotransmitters[14].

There is a need for minimizing memory impairment (MI) in epileptic patient undergoing antiepileptic treatment without compromising the quality of life. Recent reports demonstrated that combination of nootropic herbs would minimize the MI and few of them have also shown synergy of antiepileptic activity[15-18]. The goal of the current study was to assess the protective effect of nootropic herbal drink Mulmina on memory impairment (MI) induced by gabapentin (GBP) in pentylenetetrazole (PTZ) induced epileptic mouse model.

MATERIALS AND METHODS

Animals

Swiss Albino mice with 25-35g (male sex) obtained from *In vivo* Biosciences were employed in the study. The animals were housed in groups of six in a carefully monitored laboratory with a 12 h day/night cycle and the temperature was maintained at 23–27 °C. Food and water were available *ad libitum*[19-20]. Institutional Animal Ethics Committee (IAEC) of JSSCP Mysore approved to conduct all the experiments with proposal number IAEC/JSSCPM 332/2019.

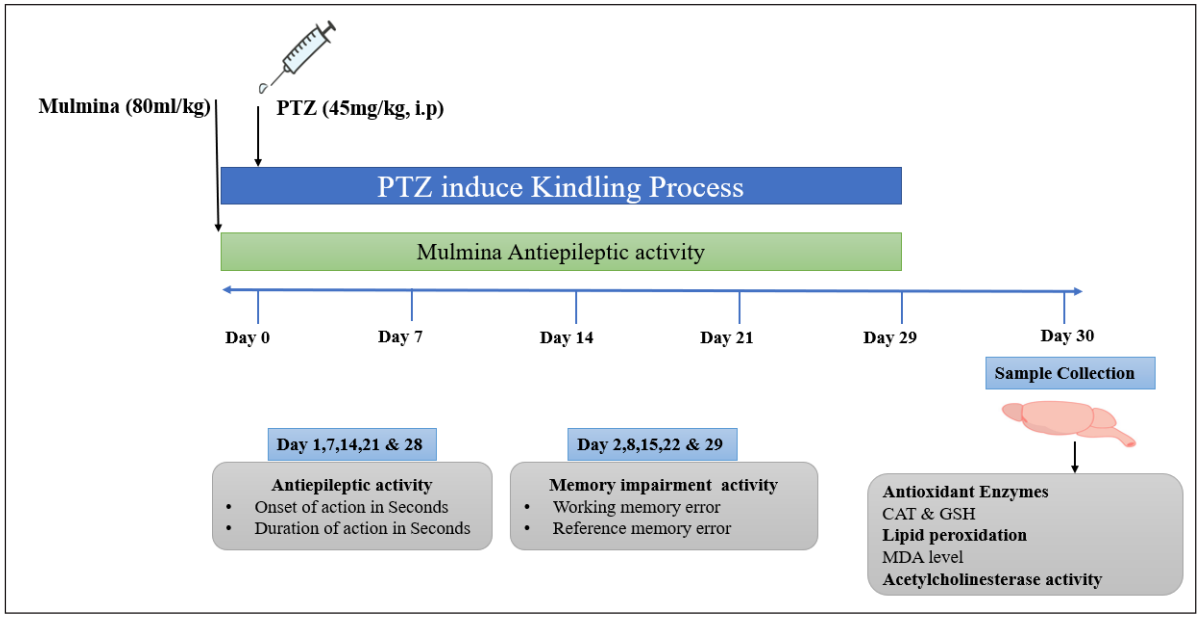

Fig. 1: Experimental flowchart elucidating the working protocol

Test sample

Mulmina™ (MUL) is a proprietary ayurvedic balya/poshak which contains 32 g of *M. indica* fruit pulp 200 mL^{-1}, 40 mg of *C. asiatica* whole plant extract 200 mL^{-1} and, 100 mg of *C. longa* 200 mL^{-1}. Other constituents include beta carotene, Vitamins B1, B2, B3, B5, B6, B9, C and E, zinc, iron and selenium.

PTZ induced epileptic mouse model

The eight groups of the animals were chosen at random (n=6): Group-I: Normal (0.5% Na CMC, p.o); Group-II: Control PTZ(45 mg kg^{-1}, i.p); Group-III: Phenytoin + PTZ (45 mg kg^{-1}, i.p); Group-IV: GBP(200 mg kg^{-1},i.p)+PTZ(45 mg kg^{-1},i.p); Group-V:GBP(100 mg kg^{-1},i.p)+PTZ(45 mg kg^{-1},i.p); Group-VI:GBP (200mg kg^{-1},i.p)+Mulmina(80 mg kg^{-1},p.o)+PTZ(45 mg kg^{-1},i.p); Group-VII:GBP(100 mg kg^{-1},i.p)+Mulmina (80 mg kg^{-1},p.o)+PTZ (45 mg kg^{-1}, i.p); Group-VIII: Mulmina(80 mg kg^{-1},p.o)+PTZ(45 mg kg^{-1}, i.p). The grouping, therapy and evaluation is displayed in Table I & Fig.1. The protective effect of MUL on MI induced by PTZ was evaluated by epileptic mouse model. The animals were treated for 29 days, antiepileptic potential of GBP in presence of MUL was evaluated on 1st, 7th, 14th, 21st and 28th days. Duration of convulsion, a time before the onset of clonic convulsions were noted[18]. Dose dependent MI activity of GBP was evaluated on 2nd, 8th, 15th, 22nd and 29th day by radial arm maze (RAM) task as well as MUL protective effect in combination was assessed. To compare the MI, phenytoin was used as standard.

Estimation of biochemical parameters in brain homogenate

Endogenous antioxidant

The endogenous antioxidants superoxide dismutase (SOD)[21], catalase (CAT)[22], reduced glutathione (GSH)[23] and membrane lipid peroxidation (MDA)[24] were assessed in the brain homogenate sample.

Acetylcholinesterase (AChE) assay

AChE activity was performed according to Ellman's method[25] and Lowry's technique was used to estimate the protein concentration[26].

Statistical analysis

Statistical1analyses-were performed using one way ANOVA followed, the TUKEY post hoc test was employed to assess the multiple comparisons between the groups. The findings are interpretated as Mean±SEM. Statistically significant was defined as a probability value of $p< 0.05$.

RESULTS

Antiepileptic activity

Antiepileptic activity of GBN at normal and reduced dose when given with/without MUL is presented in Figs. 2 and 3. The onset of convulsion was found to be significantly ($p<0.05$) more with control group of animals, which demonstrated PTZ has induced seizure in the animal. Treatment with GBP has showed significant ($p<0.05$)

decreased onset of convulsions in dose dependent manner when compared with control group. Co-administration of MUL has potentiated the activity of GBP at both doses tested and it has shown antiepileptic activity on its own (Fig. 2).

Similarly, duration of convulsions was also measured. The PTZ exposure has shown significantly higher ($p<0.05$) duration of convulsions when compared with treatment group. Exposure of GBP has demonstrated dose dependent protection against PTZ induced convulsions by

Table II: MI activity of GBP when administered alone and in combination with MUL in PTZ induced epileptic mouse tested by RAM task in PTZ epileptic mice (working memory error)

Days	Basal	Day 2	Day 8	Day 15	Day 22	Day 29
Normal	0.35±0.01	0.30±0.01	0.24±0.00	0.17±0.00	0.10±0.00	0.07±0.00
Control	0.46±0.02	0.49±0.02	1.98±0.01^{a}	2.25±0.01^{a}	2.43±0.01^{a}	2.65±0.01^{a}
PHY	0.47±0.02	0.74±0.03^{a}	4.23±0.01^{a,b}	5.13±0.05^{a,b}	6.24±0.03^{a,b}	6.89±0.12^{a,b}
GBP (ND)	0.47±0.01	0.69±0.03^{a,b}	2.31±0.05^{a,b}	3.58±0.07^{a,b}	4.44±0.10^{a,b}	5.47±0.08^{a}
GBP (RD)	0.47±0.01	0.64±0.04	1.46±0.03^{a,c}	1.94±0.03^{a,c}	2.22±0.01^{a,c}	3.15±0.04^{a,c}
GBP (ND) +MUL	0.48±0.02	0.58±0.05	0.86±0.03^{c}	0.65±0.01^{c}	0.54±0.01^{c}	0.32±0.01^{c}
GBP (RD) + MUL	0.51±0.03	0.79±0.02^{a,b}	0.87±0.02^{a,c,d}	0.56±0.02^{a,c,d}	0.19±0.00^{a,c,d}	0.18±0.01^{a,c,d}
MUL	0.47±0.02	0.44±0.02	0.36±0.03^{b,c,d}	0.34±0.07^{b,c,d}	0.15±0.00^{b,c,d}	0.09±0.01^{b,c,d}

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to normal
p<0.05, b-Significant when compared to control
p<0.05, c-Significant when compared to PHY
p<0.05, d-Significant when compared to GBP

Table III: MI activity of GBP when administered alone and in combination with MUL on RAM task in PTZ epileptic mice (reference memory error)

Days	Basal	Day 2	Day 8	Day 15	Day 22	Day 29
Normal	0.47±0.00	0.43±0.00	0.39±0.00	0.39±0.00	0.40±0.00	0.40±0.00
Control	0.47±0.00	0.59±0.00	1.24±0.03^{a}	1.45±0.01^{a}	2.11±0.02^{a}	2.45±0.01^{a}
PHY	0.49±0.00	0.63±0.01	3.34±0.05^{a,b}	4.48±0.15^{a,b}	6.72±0.08^{a,b}	7.70±0.12^{a,b}
GBP (ND)	0.44±0.00	0.57±0.00	3.68±0.08^{a}	4.44±0.10^{a,b}	5.75±0.10^{a,b}	7.18±0.03^{a,b}
GBP (RD)	0.47±0.00	0.53±0.01	1.87±0.01	2.17±0.02^{a,c}	3.60±0.04^{a,c}	4.79±0.05^{a,c}
GBP (ND)+MUL	0.49±0.00	0.52±0.00	1.34±0.03^{a,b,d}	1.19±0.02^{a,b,d}	0.97±0.02^{a,b,d}	0.70±0.01^{a,b,d}
GBP (RD)+MUL	0.43±0.00	0.45±0.00	1.28±0.01^{a,d}	1.05±0.02^{a,b,d}	0.85±0.01^{a,b,d}	0.62±0.00^{a,b,d}
MUL	0.47±0.00	0.42±0.00	0.41±0.00^{a,b,c,d}	0.35±0.00^{a,b,c,d}	0.30±0.00^{a,b,c,d}	0.21±0.00^{a,b,c,d}

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to normal
p<0.05, b-Significant when compared to control
p<0.05, c-Significant when compared to PHY
p<0.05,d-Significant when compared to GBP

significantly ($p<0.05$) decreasing duration of convulsions and it was further decreased when co-administered with Mulmina. The activity was found to be significant and same as that of standard drug PHT (Fig. 3).

Memory impairment (MI) activity

MI induced by GBP on PTZ treated epileptic mouse was evaluated by RAM task; both working and reference memories were recorded. MI was evaluated at normal as well in reduced dose of GBP to assess the dose dependent activity when administered alone and in combination with MUL.

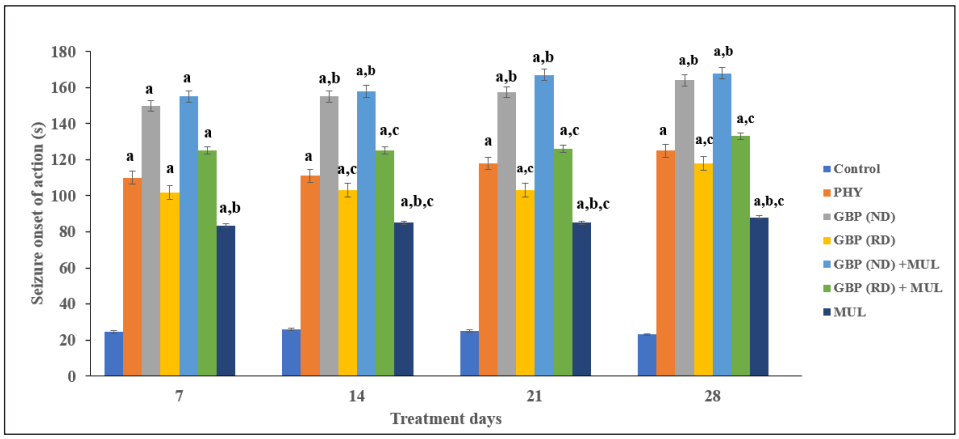

Fig. 2: Antiepileptic activity of GBP when administered alone and in combination with MUL in PTZ epileptic mice (onset of action in seconds)

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to control
p<0.05, b-Significant when compared to PHY
p<0.05, c-Significant when compared to GBP

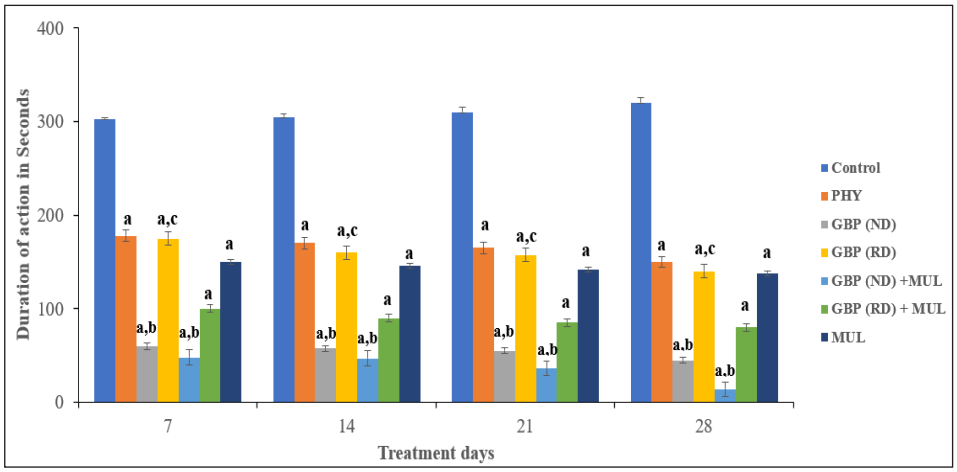

Fig. 3: Antiepileptic activity of GBP when administered alone and in combination with MUL in PTZ epileptic mice (Duration of action in seconds)

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to control
p<0.05, b-Significant when compared to PHY
p<0.05, c-Significant when compared to GBP

Working memory error (WME)

The present study shows results of working memory error caused in the temporal lobe region in the brain. Here, when the PTZ was given to control group of animals, they demonstrated significant ($p<0.05$) induction in working memory error score (0.46+0.00 to 2.65+0.08) when compared with normal group (0.03+0.01 to 0.07+0.00). Mice treated with GBP at normal and reduced dose have found significant working memory error score when compared with control group dose dependently and MI was very much similar to standard PHT. Co-administration of drink MUL has reversed MI induced by GBP at both doses tested significantly whereas MUL itself has shown memory enhancing activity (Table II).

Reference memory error (RME)

RME occurs in the temporal lobe region in the brain. Same degree of MI was observed as that of working memory error. Control group of animals shows significant ($p<0.05$) induction in reference memory error score (0.47±0.00 to 2.45±0.03) when compared with normal group (0.47±0.00 to 0.40±0.00). GBP has shown dose dependent MI by increasing the RME when compared with control group and which was reversed by co-administration of MUL at both the doses. The extent of MI induced by GBP was found to be very much similar to the standard PHT (Table III).

Table IV: MI activity of GBP when administered alone and in combination with MUL in PTZ epileptic mice (SOD, GSH, TBARS, CAT estimation)

Group	SOD (µg mg^{-1} of protein)	GSH (µg mg^{-1} of protein)	TBARS (µg mg^{-1} of protein)	CAT (µg mg^{-1} of protein)
Normal	17.18±0.26	15.92±0.25	0.84±0.01	15.96±0.06
Control	10.66 ± 0.18^{a}	11.37 ± 0.11^{a}	16.22 ± 0.20^{a}	8.01 ± 0.05^{a}
PHY	$8.04\pm0.05^{a,b}$	$8.62\pm0.12^{a,b}$	$18.15\pm0.06^{a,b}$	$8.83\pm0.08^{a,b}$
GBP (ND)	$5.09\pm0.07^{a,b,c}$	$4.55\pm0.14^{a,b,c}$	$20.91\pm0.18^{a,b,c}$	$5.46\pm0.10^{a,b,c}$
GBP (RD)	$7.03\pm0.11^{a,b,d}$	$6.20\pm0.07^{a,b,c,d}$	$15.39\pm0.06^{a,b,d}$	$6.60\pm0.08^{a,b,c,d}$
GBP (ND)+MUL	$10.97\pm0.19^{a,b,c,d}$	$7.11\pm0.03^{a,c,d}$	$9.83\pm0.15^{a,b,c,d}$	$7.54\pm0.13^{a,c,d}$
GBP (RD)+MUL	$13.50\pm0.19^{a,b,c,d}$	$8.12\pm0.04^{a,b,c,d}$	$5.48\pm0.06^{a,b,c,d}$	$8.42\pm0.04^{a,b,c,d}$
MUL	$15.13\pm0.34^{b,c,d}$	$13.02\pm0.12^{a,c,d}$	$1.70\pm0.11^{a,b,c,d}$	$13.22\pm0.10^{a,c,d}$

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to normal
p<0.05, b-Significant when compared to control
p<0.05, c-Significant when compared to PHY
p<0.05, d-Significant when compared to GBP

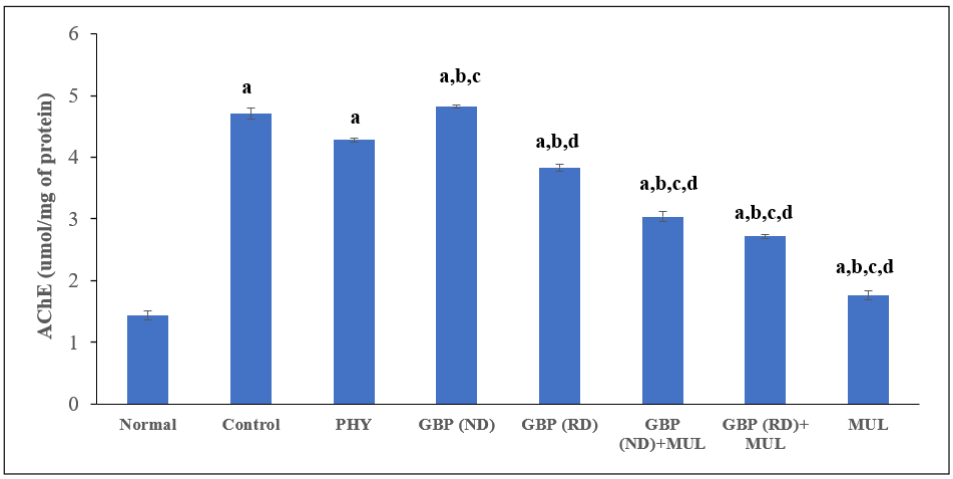

Fig.4: MI activity of GBP when administered alone and in combination with MUL in the PTZ epileptic mice (AChE estimation)

Value are expressed as Mean ± SEM, n=6
p<0.05, a-Significant when compared to normal
p<0.05, b-Significant when compared to control
p<0.05, c-Significant when compared to PHY
p<0.05, d-Significant when compared to GBP

Endogenous antioxidant activity

To assess the level of antioxidant enzymes in the brain homogenate after exposure to GBP and protective effect of MUL, various antioxidant enzymes like SOD, GSH, CAT and TBARS were determined at the end of the experimental protocol.

SOD: Animals treated alone with PTZ have shown significantly ($p<0.05$) reduced amounts of SOD when compared with normal group of animals. This demonstrates that PTZ induced convulsions deteriorate the brain by oxidative stress, whereas administration of GBP and PHT further damage by decreasing the SOD level. Herbal drink MUL has shown a significant ($p<0.05$) protective effect by increasing SOD level when given alone as well as in combination with GBP (Table IV).

GSH: Control group have reduction of GSH level when compared with normal group and observations are very much similar to SOD level. As explained above, administration of GBP significantly ($p<0.05$) decreased the GSH level in dose dependent manner, whereas MUL reverses GSH level (Table IV).

CAT: Control group has reduction of CAT level when compared with normal group and observations are very much similar to GSH level. It was evident that

administration of GBP has produced oxidative stress dose dependently by significantly ($p<0.05$) decreasing GSH level, whereas MUL treatment has shown protective effect (Table IV).

TBARS: Control group has shown significantly ($p<0.05$) increased TBARS level more than the normal group, which demonstrates the oxidative stress. GBP still increases the TBARS level dose dependently, whereas MUL co-administration has shown the protective effect (Table IV).

AChE activity

Control group has resulted in increased AChE level when compared to normal group, which shows decreased level they of acetylcholine. When animals were treated with GBP they showed a significant (*p<0.05*) increase in AChE level dose dependently compared with control group. GBP at both dose levels, exhibited same degree of effects as that of standard PHT, whereas, MUL reverses the AChE level. MUL itself has shown AChE level very much similar to normal group, which demonstrates the nootropic activity (Fig. 4).

DISCUSSION

An uncontrolled electrical spark in the brain is a seizure and epilepsy is more than double the frequency of a seizure. Epilepsy is a persistent brain condition that induces psychotic phenomena, unconsciousness and irregular body movement. At present, it primarily affects children and elderly patients; epilepsy is also treated with the use of possible AEDs. MI is one of the biggest disadvantages due to some of the risk factors in the body that are adversely affected by epilepsy as well as AEDs. GBP is a gamma-amino butyric acid structural analogue (GABA). Among most drugs for treating partial seizures, it is one of the most effective anticonvulsant agents.

MOA is primarily involved in potentiating the release of GABA, showing a decrease in the absorption of branch-chain amino acids into neurons, and hence a decrease in the production of neuronal glutamate. The amount of AChE that contributes to cognitive dysfunction can be decreased by GBP. Test sample MUL is a patented ayurvedic balya/poshak containing 32 g of fruit pulp 200 mL^{-1} of *M. indica*, 40 mg of whole plant extract 200 mL^{-1} of *C. asiatica* and 100 mg of *C. longa* 200 mL^{-1}. MUL may show changes in cognitive dysfunction stimulated by antiepileptic drugs and increase the effectiveness of GBP.

PTZ is an epileptogenic agent which causes sudden lapses in attention that usually appears in children. As PTZ is a non-competitive antagonist of GABA receptor, it is specifically assessing the excitability of the CNS and GABA activity. Mice were observed for seizures especially clonic/tonic, tonic and clonic after administration of PTZ through i.p. route. The seizure was assessed for 30 minutes and evaluated every 10 minutes for signs and symptoms like abnormal muscle movements, immobility and loss of consciousness. Parameters – onset of convulsion and duration of convulsion.

PTZ (an antagonist of GABA which is not competitive) is mainly utilized to induce seizures and investigate CNS excitability and GABA behaviour. PTZ was administered 1 h before the treatment. The anti- epileptic activity was determined by the Racine Scale. The GBP (ND) (200 mg kg^{-1}) was showing less epileptic activity compared to PHY. GBP (RD) (100 mg kg^{-1}) was not found to decrease as compared to the GBP (ND). Combinational drugs such as GBP (ND) and MUL (80 mL kg^{-1}) significantly reduced the convulsion activity when compared with control, PHY, GBP (RD) + MUL. MUL demonstrates better action along with GBP to treat epilepsy. The technique used to get a smaller grade of MI, which is by reducing the dosage of AED, has created an unfortunate consequence of lower efficacy.

RAM was useful to determine the working and reference memory impairment in the PTZ induced epilepsy. Here, RAM and MI error scores are proportionate to one another, that is induced errors in RAM task are the indication of induced MI in the brain and vice versa. This is the fundamental principle used to evaluate the findings of RAM. The outcome of the present research work are expressed in Tables II and III.

When PTZ was administered to the control group, the result was the enhancement of WMI when compared with a normal group where it indicates the cognitive impairment due to PTZ administration. Consequently, the statement that MI is correlated with TLE is authenticated[27]. When GBP (ND) (200 mg kg^{-1}, i.p) was administered to mice, again enhancement of WMI compared with the control group, PHY as well as GBP (RD) (100 mg kg^{-1}) was observed[28]. It justifies the reality about AEDs, it has its own ability to damage memory in addition to exacerbating MI's condition[29]. GBP (ND) (200 mg kg^{-1}, i.p) + MUL (80 mL kg^{-1}, p.o) were given to the animals; it showed significant reduction in WMI when compared with GBP (RD) (100 mg kg^{-1}, i.p) + MUL (80 mL kg^{-1}, p.o.)., that helps to reducing the MI level by reducing the administrative dose. Along with this, MUL (80 mL kg^{-1}, p.o.) alone was

administered which showed a significant reduction in the WMI when compared with the control group. When PTZ was administered to the control group, the result was an enhancement of RMI when compared with a normal group where it indicates the cognitive impairment due to PTZ administration[30]. When GBP (ND) (200 mg kg^{-1}, i.p) was administered to mice, again enhancement of RMI differentiated with the control group, PHY as well as GBP (RD) (100 mg kg^{-1}). It is representing the MI property due to GBP[27]. GBP (ND) (200 mg kg^{-1}, i.p)+MUL (80 mL kg^{-1}, p.o) were given to the animals; it showed significant reduction in RMI when differentiated with GBP (RD) (100 mg kg^{-1}, i.p)+MUL (80 mL kg^{-1}, p.o.). Along with these doses, MUL (80 mL kg^{-1}, p.o.) alone was administered which showed a significant reduction in the RMI when differentiated with the control group[31].

AChE is a cholinergic enzyme responsible for the metabolism of the neurotansmitter acetylcholine. Discovery of new AChE inhibitors prevents the breakdown of acetylcholine[32]. Due to high level of AChE, most of the normal and reduced doses have shown a negative memory effect. The explanation is thtat; increase in GBP may be through oxidative stress which increases AChE production, therefore reducing the acetylcholine level in the brain causing significant MI[33]. The outcome of the present research work on AChE is expressed in Fig. 4, when PTZ was used to induce epilepsy and it resulted in enhanced the level of AChE when compared with the normal group. Treatment with GBP both doses and PHY also demonstrated high level of AChE when compared with PTZ non treated animals. MUL reverses the elevated level of AChE when combined with both the doses of GBP; this demonstrates the protective effect of MUL in MI induced by GBP.

Thus, utilization of Mulmina™ as combinational drugs with AEDs reduces the dose of the synthetic AEDs by providing synergistic activity and also reduces the range of adverse effects caused by the AEDs to the human body. Our analysis gives a forum for more comprehensive nootropic work to correct MI caused by AEDs. We have observed swellings in the right limb of all the animals treated with GBP (ND) + MUL. Whereas only few animals treated with GBP (RD) + MUL have shown swellings in right hind limb. Hence, further research can be done to determine the exact reason or mechanism behind this adverse event shown by the combination therapy.

CONCLUSION

The findings of the present study revealed the MI potential of GBP and correction by co-administration of Mulmina™. However, further study is required to establish the molecular mechanism involved in MI caused by GBP.

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