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Research Article

ANTICONVULSANT ACTIVITY OF MAHAKALAYANAKA GHRITA IN MAXIMAL ELECTRO-SHOCK AND PENTYLENETETRAZOLE INDUCED SEIZURES IN RATS

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ABSTRACT

The present study was to evaluate the anticonvulsant activity of Maha Kalayanaka Ghrita on maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizures in rats. The ayurvedic formulation, Maha Kalayanaka Ghrita (MKG) was subjected to acute toxicity and then screened anticonvulsant activity on MES and PTZ induced seizure models. In addition the brain neurotransmitters levels were estimated. Acute toxicity of test drug was non-toxic upto the recommended dose 2000mg/kg p.o. Animals were pretreated with MKG at doses 200 & 400 mg/kg for 14 days. Study results showed reduction of extensor phase of MES induced convulsion and prolongation of onset and reduction of duration of convulsion in PTZ model. Furthermore, MKG had reduced the level of excitatory neurotransmitters noradrenaline, glutamate and serotonin and increased the level of inhibitory neurotransmitters such as Gamma-aminobutyric acid and dopamine as compared to untreated rats. Hence it concluded that MKG possesses anticonvulsant activity and potential effect on neurotransmitters levels in the brain.

Keywords: Anticonvulsant activity, Neurotransmitters, Maha Kalayanaka Ghrita, Acute toxicity & Seizures.

INTRODUCTION

Epilepsy is a neuropsychological disorder, affecting more than 50 million people worldwide[1]. Disturbance of naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters in central nervous system are assumed to be the main cause of convulsive episodes[2]. There are number of drugs available for the treatment of epilepsy in modern therapy. But the major disadvantages being faced in their side effects and chronic toxicity. One patient out of three is resistant to antiepileptic drug[3] thus, there is a need for new drugs which have least side effects and minimum interaction and provide more effectiveness. MahaKalayanaka Ghrita (MKG), an ayurvedic polyherbal formulation that is in the form of herbal ghee form. The ingredients are mentioned below. [Table1] It is traditionally used as to treat epilepsy. No scientific evidences were available to prove their efficacy. The present study is focussed on the evaluation of anticonvulsant acitivity of Mahakalayanaka Ghrita in maximal electro-shock and pentylenetetrazole induced seizures in rats. In addition the neurotransmitters such as Noradrenaline (NA), Dopamine (DA), serotonin (5-HT), Gamma Amino Butyric Acid (GABA) and glutamate (GLU) levels were estimated.

Table 1: Ingredients of Mahakalayanaka Ghrita

Ingredients	Botanical Name
SARIVA	*Hemidesmus indicus*
HARIDRA	*Curcuma longa*
DARUHARIDRA	*Berberis aristata*
SHALAPARNI	*Desmodium gangeticum*
PRISHNAPARNI	*Uraria picta*
PHALINI	*Callicarpa macrophylla*
TRIPHALA	*Terminalia chebula*
AMLA	*Emblica officinalis*
VISHALA	*Citrulus cholocynthis*
BHADRA	*Amomum subulatum*
ELA	*Cedrus deodara*
DEVADARU	*Prunus avium*
ELAVALUKA	*Valeriana wallichi*
NATA	*Solanum indicum*
BRIHATI	*Saussurea lappa*
KUSHTA	*Rubia cordifolia*
MANJISHTA	*Mesua ferrea*
NAGAKESHARA	*Punica granatum*
DADIMAPHALATWAK	*Embelia ribes*
VELLA	*Abbies webbiana*
TALISAPATRA	*Eleteria cardamom*
ELA	*Jasminum sambac*
MALATI	*Nymphea stellata*
UTPALA	*Baliospermum montanum*

MATERIALS AND METHODS

Animals

Albino Wister rats (150-200 g) of either sex were used. Animals were housed in groups of 6-8 per cage at a temperature of $25^0 \pm 1^0$C and relative humidity of 45-55%. A 12: 12 dark: light cycle was followed during the experiments. Food consisted of normal rat chow and water was provided *ad libitum*. They were fasted overnight before the experiments and were transferred to the laboratory at least 1 h before the start of the experiment. The present study was carried out during 09:00- 16: 00 h. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg No: 991/PO/C/06/CPCSEA)

Drugs

The test drug Maha Kalayanaka Ghrita (Arya vaidya sala, Kottakkal, India), Phenytoin and diazepam (IDPL, Chennai, India), Pilocarpine (FDC, Mumbai, India), lithium sulphate (Glenmark Laboratories, India), Atropine sulphate injection I.P. (Martin and Brown Pharmaceticals) Pentylene tetrazole and strychnine were purchased from Modern Scientific (Nashik, India) was used for the study.

Acute oral toxicity study

The acute toxicity of MahaKalayanaka Ghrita was determined as per the OECD guildeline no.423 (Acute Toxic Class Method).[4]It was observed that the test drug was not mortal even at 2000mg/kg dose. Hence, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of this dose were selected for further studies.

Experimental study design

The animals were randomly divided into four groups each composed of six animals as follows.

Maximal electroshock (MES) induced convulsions

Group I: Control (received 1% w/v gum acacia solution, p.o.)
Group II: Phenytoin (25 mg/kg, p.o.)
Group III: MKG (200 mg/ kg, p.o.)
Group IV: MKG (400 mg/kg, p.o.)

All the treatments were carried out once daily for 14 days. On the 14th day of the drug treatment, after 1hour, seizures were induced to all the groups of animals by using Electroconvulsiometer (150 mA current for 0.2 sec). The duration of various phases of convulsion was observed by giving importance to hind leg tonic extension.[5, 6]

Pentylenetetrazole (PTZ) induced convulsion

In present study in all four groups of animals, the drug pre-treatment schedule was once daily for 14 days.

Group I: Control (received 1% w/v gum acacia solution, p.o.)
Group II: Diazepam (4 mg/kg, p.o.)
Group III: MKG (200 mg/ kg, p.o.)
Group IV: MKG (400 mg/kg, p.o.)

On the 14th day, PTZ (90mg/kg body weight, *s.c*) was administered to all the groups to induce clonic convulsions. Animals were observed for a period of 30 mins post – PTZ administration.Ability to delay the onset and duration of clonic convulsion was noted and percentage protection of animals from mortality was recorded[7, 8]

NEUROTRANSMITTERS LEVEL[9, 10]

Preparation of brain tissue extracts

On the 14th day after observed the convulsion all groups rats were sacrificed, whole brain was dissected and separated the forebrain. Weighed quantity of tissue was homogenized in 5ml HCl–butanol for about 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (1 ml) was removed and added to centrifuge tube containing 2.5 ml heptane and 0.31ml HCl of 0.1 M. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions as above in order to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2 ml) was taken for Gama –Aminobutyric Acid (GABA), Glutamate (GLU), Serotonin(5-HT), Nor adrenaline (NA) and Dopamine (DA) assay. All steps were carried out at 0^0C.

Nor-adrenaline and dopamine Assay

To the 0.2 ml of aqueous phase, 0.05 ml 0.4 M HCl and 0.1 ml of Sodium acetate buffer (PH- 6.9) were added, followed by 0.1 ml iodine solution (0.1 M in ethanol) for oxidation. The reaction was stopped after 2 min by addition of 0.1 ml Na_2SO_3 solution. 0.1 ml Acetic acid is added after 1.5 min. The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-375 nm for dopamine and 395-485 nm for nor-adrenaline.

Serotonin and Glutamate Assay

To 0.2 ml aqueous extract 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at for serotonin360-470 nm and Glutamate 515 nm in the spectrofluorimeter.

GABA Assay

A sample (0.1ml) of tissue extract was placed in 0.2ml of 0.14 M Ninhydrin solution in 0.5M corbonate-bicorbonate 1 buffer (pH9.95), kept in a water bath at 60°C for 30min, then cooled and treated with 5ml of copper tartar ate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10min fluorescence at 377/455nm in a spectofluorimeter was recorded.

Statistical analysis

The results of anticonvulsant activity are expressed as mean ± SEM from six animals in each group. Results were statistically analysed using (Graphpad Prism Software version 6.01) by one- way ANOVA method followed by Tukey's multiple comparison tests.

RESULTS

Effect of MKG formulation on MES induced Seizures

The duration of tonic hind leg extension in rats treated with vehicle was 24±1.32 seconds whereas Phenytoin treated animal's exhibits abolished tonic hind leg extension. The pretreatment of MKG at doses of 200 mg/kg and 400 mg/kg protected animals from seizures and significantly ($P< 0.001$) reduced the duration of tonic hind leg extension to 9.50±0.76 and 4.33± 1.43 seconds respectively. The results were given in (Table 2).

Table 2: Effect of MKG formulation on MES induced convulsion

Treatment Group	Various phases of convulsions (seconds)				
	Flexion	Extension	Clonus	Stupor	Recovery
Control (1% gum acacia p.o)	9 ± 0.73	24 ± 1.32	29.67 ± 1.38	36 ± 1.81	156.3 ± 4.32
Phenytoin (25 mg/kg, p.o)	4 ± 0.86**	0***	18.50 ± 1.17***	19 ± 1.24***	86.17 ± 1.66***
MKG (200 mg/kg, p.o)	7.50 ± 0.77*	9.50±0.76***	21.17 ±1.30***	28.83 ± 1.30*	117.3 ± 3.17***
MKG (400 mg/kg, p.o)	4 ± 0.87**	4.33 ± 1.43***	17.83 ± 1.95***	18.83 ± 1.54***	89. 83 ± 2.55***

Values are expressed as mean ± SEM; n= 6; one-way ANOVA followed by Tukey's Multiple Comparison Test, *P<0.05; **P< 0.01, ***P< 0.001 Vs Control.

Effect of MKG formulation on PTZ induced seizures

In rats treated with vehicle, the onset of convulsion appeared at 37.17± 3.61 seconds after PTZ and all rats died after seizures. Diazepam (4mg/kg, p.o.) treated animals completely abolished the convulsion (P<0.001) and 100% protection of mortality. The MKG (200 mg/kg) delayed the onset of convulsion at 131.7±6.15 (P<0.001) seconds and have shown 66.66% protection of mortality whereas MKG (400 mg/kg) treated animals completed abolished the convulsion (P<0.001) and have shown 100% protection from mortality. The results were given in Table 3.

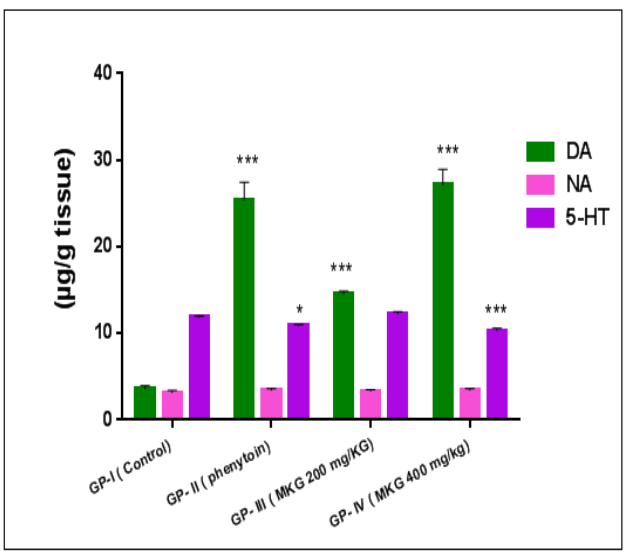
Table 3: Effect of MKG formulation on PTZ induced convulsion

Drug treatment	Convulsion (seconds)			Status (or) No of animals alive/ no of animals used	% protection of mortality
	Onset	Duration	Nature and severity		
Control (1% gum acacia p.o)	37.17± 3.61	300 ± 11.55	Jerky movement, Straub tail and convulsion	0/6	0
Diazepam (4 mg/ kg, p.o)	0***	0***	-	6/6	100
MKG (200 mg/kg, p.o)	131.7± 6.15***	118.33±7.60***	Jerky movement and convulsion	4/6	66.66
MKG (400 mg/kg, p.o)	0***	0***	-	6/6	100

Values are expressed as mean ± SEM; n= 6; one-way ANOVA followed by Tukey's Multiple Comparison Test, ***P< 0.001 Vs Control.

Effect of MKG formulation on neurotransmitters level in MES induced seizures:

In MES test, DA level significantly (P< 0.001) increased in phenytoin and MKG treated groups as compared to control group. Phenytoin and MKG treated groups showed insignificant effect in NA level as compared with control group. GABA level significantly (P<0.001) increased in phenytoin and MKG 400 mg/kg treated group whereas MKG at the dose of 200mg/kg have shown insignificant effect. Phenytoin and MKG 200 mg/kg treated animals shown insignificant variation in GLU level whereas MKG 400 mg/kg significantly decreased the GLU level when compared with control. 5-HT level significantly (P< 0.05) decreased in standard group whereas MKG (200 mg/kg) showed insignificant result but the MKG at the dose of 400 mg/kg significantly (P<0.001) decreased when compared to control group.



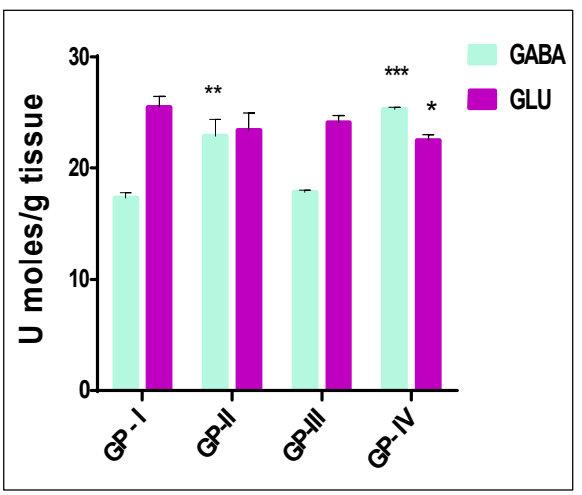


Figure 1: Effect of MKG formulation on neurotransmitters level in MES induced seizures:
***P <0.05; ****P< 0.01, *****P< 0.001 Vs control**

Effect of MKG formulation on Neurotransmitters level in PTZ induced seizures

In PTZ test, DA level significantly (P< 0.001) increased in diazepam treated rats whereas MKG (200 mg/kg) showed insignificant effect but the MKG (400 mg/kg) have significantly (P<0.001) increased the DA level as compared with control group. MKG 200 & 400 mg/kg treated animals shown insignificant variation in NA level when compared with the control. GABA level significantly increased in diazepam and MKG (400mg/kg) treated groups but the MKG at the dose of 200mg/kg have shown insignificant result when compared to control group. GLU level showed significantly (P<0.001) decreased in diazepam and MKG treated groups as compared with control. 5-HT level significantly (P< 0.001) decreased in standard group and MKG treated groups when compared with control group

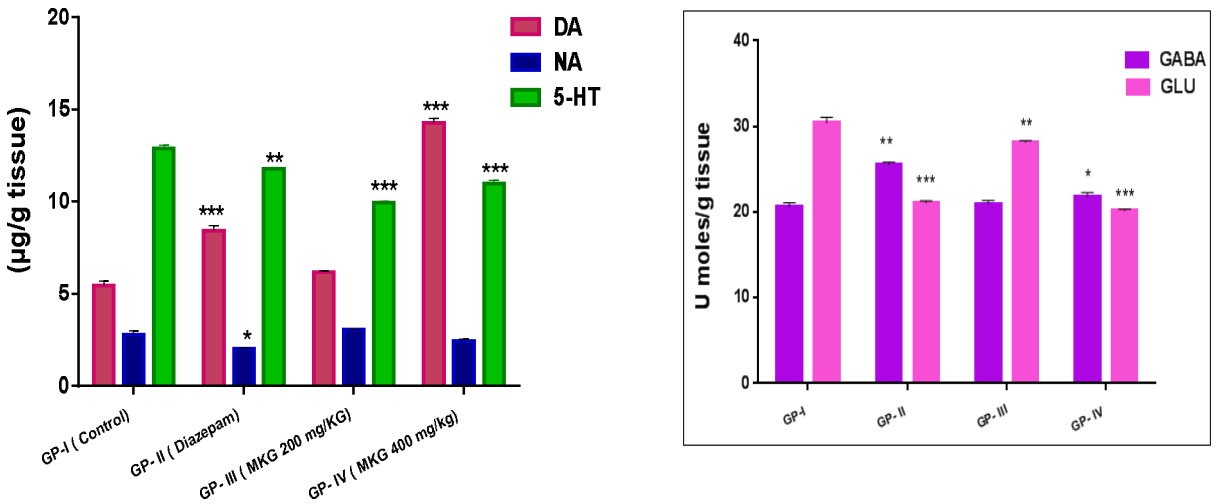


Figure 2: Effect of MKG formulation on Neurotransmitters level in PTZ induced seizures
*P <0.05; **P< 0.01, ***P< 0.001 Vs control.

DISCUSSION

The most popular and widely used animal seizures models are MES and PTZ. MES test correlates with efficacy in suppressing generalized tonic-clonic and partial seizures.[11] MES induced tonic extension can be prevented by AED that inhibit either voltage gated channels or by blocking glutamergic excitation mediated by the N-methyl-D-aspartame receptor.[12] PTZ- induced seizures test is considered as an experimental model for generalized absence seizure.[13] It is widely recognized that PTZ leads to tonic-clonic seizures by suppressing the inhibitory effects of GABAergic transmission.[14] In present study possess pretreatment of MKG in MES test significantly inhibited generalized tonic- clonic seizures as evidenced by decrease duration of tonic hind limb extension and PTZ-induced seizure animal model significantly inhibited generalized absence and myoclonic seizures as evidenced by abolition of tonic extensor jerks of hind limbs was evaluated.

An imbalance between the Excitatory and Inhibitory Neurotransmitters is responsible for seizures.[15] GABA is major inhibitory neurotransmitters of CNS and increase in its level in brain has variety of CNS dependent effects including anticonvulsant effect. In the present study indicates that have shown significant increase in GABA level in MES and PTZ induced seizures.

The role of dopamine in the pathophysiology of focal epilepsy is controversial. The dopaminergic influence on epileptic seizures araising from mesial temporal structure might be inhibitory (i.e., via dopaminergic hippocampal projection enhancing a Ca^{2+} - dependent K+ conductance).[16] In present study, MKG significant increase in DA level in both validated animal models. From this result it is concluded that MKG might have possible action on DA receptors and also blocked Ca^{2+} - dependent K^{+} conductance.

Glutamate is the principle excitatory NT in the brain. It also plays a critical role in epileptogenesis. The process of "Kindling" limbic seizures in rodents by repeated electrical stimulation is dependent on activation of NMDA receptors.[17] The hypothesis is appealing: when glutamate transport is blocked, excess glutamate accumulates in the synaptic space, leading to increased NMDA –receptor activation, further glutamate release, and seizures. And some infantile epilepsy might be releated in part, to glutamate transporter dysfunction. One important future step is to show that enchancement of glutamate transporter function can protect animals from seizures.[18]In present study, MKG have shown significant decrease in glutamate level in both animal models from this results it is concluded that MKG might have acted on glutamate transporters and inhibited the glutamate mediated excitatory effects by blocking NMDA receptor. Evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures[19] MKG have shown significant decrease in 5- HT level when compared with control groups in both animal models. So, it is concluded that the MKG might have acted on 5-HT nerve ending.

The vagus nerve stimulation (VNS) induces an increase in the extracellular hippocampal concentration of noradrenaline, but not of dopamine, serotonin and GABA; VNS prevents the development of pilocarpine-induced limibic seizures only in those rats with VNS – induced increases in hippocampal NA of at least 70%; and that selective α_2- adrenoreceptor antagonism in proximity of the seizures focus abolishes the seizures-suppressing effect of VNS. Taken together, these findings provide convincing evidence for the existence of a strong causal link between increased nor-adrenaline signaling and the anticonvulsant effect of VNS.[20] In present study, MKG have shown insignificant effect on NA level when compared with control groups in both models. We observed significant reduction in NA level; So MKG might have acted on cholinergic nerve ending but not on adrenergic nerve ending.

CONCLUSION

In present study, the result of MKG possesses anticonvulsant activity against maximal electroshock and pentylenetetrazole-induced convulsions. Furthermore, MKG possess potential effect on neurotransmitters level in the brain.

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