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EVALUATION OF ANTI-SEIZURE ACTIVITY OF ETHANOLIC EXTRACT OF MUSA PARADISIACA FLOWER ALONE AND IN COMBINATION WITH ANTI-SEIZURE DRUGS IN SWISS ALBINO MICE

M. S. Upadhyay [*1] and B. M. Purohit [2]

Department of Pharmacology [1], Dr. M. K. Shah Medical College and Research Centre Smt. S. M. S. Multispeciality Hospital, Chandkheda, Ahmedabad - 382424, Gujarat, India.
Department of Pharmacology [2], Government Medical College Bhavnagar - 364001, Gujarat, India.

Keywords:

MES (Maximal Electroshock), *Musa paradisiaca*, Pentylenetetrazole (PTZ), Phenytoin, Seizures, Sodium Valproate, Swiss albino mice

Correspondence to Author:
Dr. Mitul Sanjaybhai Upadhyay

Assistant Professor,
Department of Pharmacology,
Dr. M. K. Shah Medical College and Research Centre Smt. S. M. S. Multispeciality Hospital, Chandkheda, Ahmedabad - 382424, Gujarat, India.

E-mail: drmitulupadhyay@gmail.com

ABSTRACT: Background: There are numerous anti-seizure drugs on the market, but side effects at the highest dosage that can be tolerated account for the majority of prescription discontinuations. Research on *Musa paradisiaca's* synergistic activity is of utmost importance in getting insight into its anti-seizure potential. **Methods:** Anti-seizure activity of *Musa paradisiaca* on generalized tonic-clonic seizure was evaluated using standard maximal electroshock seizure method and on absence seizure using pentylenetetrazole test. Both models had 6 groups each: vehicle control, active control, high dose herb, low dose herb, combination of active control (sub-therapeutic dose) with low dose herb & combination of active control (sub-therapeutic dose) with high dose herb. **Results:** In MES model *Musa paradisiaca* in high dose prevented both tonic extension and tonic flexion. In PTZ model *Musa paradisiaca* in high dose and low dose significantly increase the latency of onset of seizures and decrease the duration of seizure compared to vehicle control. There was no significant effect on onset of 1st myoclonic jerk. Addition of *Musa paradisiaca* to the sub-therapeutic dose of sodium valproate and phenytoin showed a synergistic effect. **Conclusion:** Inhibition of seizure by *Musa paradisiaca* could be due to the presence of flavonoids that act as a partial positive allosteric modulator at GABAA (γ-aminobutyric acid) receptors, penetrate the blood-brain barrier and possess the anti-convulsant activity and also because of its antioxidant property. *Musa paradisiaca* can be effective alone or as add-on in generalized tonic-clonic seizures and as add-on with sodium valproate for absence seizures.

INTRODUCTION: Brief episodes of involuntary movement that may involve a part of the body (partial) or the entire body (generalized) that are sometimes accompanied by loss of consciousness and control of bowel or bladder function are called seizure.

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Epilepsy is characterized by recurrent (two or more) seizures which are a result of excessive electrical discharges in a group of brain cells.

Seizures can vary from the briefest lapses of attention or muscle jerks to severe and prolonged convulsions and also in frequency, from less than one per year to several per day, according to a fact sheet on epilepsy by the World Health Organization (WHO). Currently, around 50 million people worldwide live with epilepsy out of which 80% reside in low to middle-income countries and one-sixth in India [1]. Management of patients with epilepsy needs constant treatment with which

almost 70-80% of them achieve good control. Most of these drugs require therapeutic drug monitoring (TDM) due to their narrow therapeutic margin. Around 20% of patients develops therapeutic failure with a tolerated dose. Many of these drugs have potential drug interaction [2, 3]. Adverse effect is a leading cause of treatment failure or treatment discontinuation in people with epilepsy. Most of the anti-epileptic drugs produce dose-dependent adverse drug reactions which can be reduced by using sub-therapeutic doses with the new compounds which act synergistically with already approved anti-epileptic drugs [4, 5]. Thus major problems with anti-epileptic drugs like adverse effects, drug interaction, compliance and need for long-term treatment and TDM commences further research to find newer drugs.

Musa paradisiaca is widely known for its antioxidant effect due to the presence of flavonoids [6]. *Musa paradisiaca* is used because of its antilithiatic, antioxidant, antibacterial, antidiabetic, antiulcer, antidiarrhoeal, hypocholesterolaemic, hepatoprotective, antisnakevenom, wound healing, hair growth promoting, antifungal and antimenorrhagic activity [7]. Its flower is used for dysentery, bronchitis, ulcer, anemia, malaria, heart pain, stomach cramps and diabetes [8, 9]. Ethanolic extract of *Musa paradisiaca* in addition to flavonoids contains vitamin c, vitamin e, tocopherol, alkaloids, tannin, saponins and glycosides [8]. The rationale behind choosing ethanol extract was that ethanol extract demonstrated stronger antioxidant activity in free radical scavenging assay than aqueous extract and flavanoid which are responsible for anti epileptic activity of *Musa paradisiaca* flower were absent in aqueous extract [7, 8]. Anti-seizure activity of *Musa paradisiaca* stem is already established in PTZ model in rats [10]. *Musa sapientum* (banana) another variety from same species and family also showed anti-epileptic activity [11]. In 'medicinal book of herbs' and some other articles; it has been said that *Musa paradisiaca* possesses an antiepileptic effect [12.] This study was planned to evaluate the antiepileptic effect of oral administration of Ethanolic extract of *Musa paradisiaca* flower in low and high doses and its effect with sub-therapeutic dose of standard drugs phenytoin and sodium valproate in two different chronic models (14 days) in Swiss albino mice.

MATERIALS AND METHODS:

Experimental Animals: Swiss albino mice (mus musculus), 3-4 months of age (18 to 34 g) of either gender were procured from the central animal house of the institute. They were housed in standard transparent polypropylene cages (25cm×19cm× 13cm) with wheat husk bedding kept under controlled room temperature (26 ± 3 ºC) and humidity (40 ± 5%) in a 12 hours light/12 hours dark cycle. The animals were kept on a standard laboratory diet and water *ad libitum*. All the animals were acclimatized to the laboratory conditions at least one hour before the experiments. Food was withdrawn 12 hrs before the experiments. The mice were handled with care as per internationally accepted guidelines and norms for handling and care of animals, as provided by CPCSEA, India and good laboratory practice (GLP) guidelines.

Drugs and Chemicals: *Musa paradisiaca* was procured from Kuber Impex Ltd., Indore, Madhya Pradesh, India. Free samples of analytical grade Sodium Valproate (Pure and Cure Private Limited, Haridwar Uttarakhand), Phenytoin and Pentylenetetrazole (Sigma Aldrich, Bangalore, India) were used in the study.

Acute Oral Toxicity Study: An acute toxicity assessment was conducted in accordance with the guidelines stipulated by the Organization for Economic Cooperation and Development (OECD 423). The selection of the initial dose level followed the protocol, with options comprising four predetermined levels: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight. Subsequent to dosing, female Swiss albino mice were subjected to individual monitoring for overt behavioral, neurological, autonomic, and toxic manifestations. Observations were recorded at intervals, including at least one assessment within the initial 30 minutes, periodic evaluations over the initial 24 hours (with particular emphasis on the first 4 hours), and daily surveillance extending to a total duration of 14 days. Remarkably, *Musa paradisiaca* demonstrated non-toxicity, even when administered at the highest dose of 2000 mg/kg. Consequently, for the current investigation, two doses of *Musa paradisiaca* (100 mg/kg and 200 mg/kg) were selected for the evaluation of their anti-seizure activity.

Experimental Study Design: The anti-seizure activity of *Musa paradisiaca* and its combination with standard drug (phenytoin for tonic-clonic seizure and sodium valproate for absence seizure) was evaluated by maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizures respectively after chronic dosing of either herb/standard drug/normal saline for 14 days in mice. Both procedures were started 60 min after oral treatment on day 14 with the test compound or the vehicle. Recordings were done for both experiments and were later analyzed by a rater who was blinded to the treatment. Each group contained 8 animals (4 male-4 female) and animals were randomized using Rando software.

MES Induced Seizure: MES test was performed as catalogued by Giardina and Gasior in 2009. Animals were divided into six groups, each group having eight animals. The route of administration was oral for all groups. Different group received different treatment for 14 days. Group 1(vehicle control) received Normal saline. Group 2(active control) received Phenytoin (50mg/kg). Group 3(low dose herb) and Group 4(high dose herb) received *Musa paradisiaca* in the dose of 100 mg/kg and 200mg/kg respectively. Group 5 (sub-therapeutic active control+ high dose herb) was given Phenytoin (25mg/kg) + *Musa paradisiaca* (200mg/kg) and Group 6(sub-therapeutic active control+ low dose herb) was given Phenytoin (25mg/kg) + *Musa paradisiaca* (100mg/kg). After 60 min of the last oral dose on 14^{th} day shock of 123mA intensity was given to mice for 0.2 seconds using Digital Electroconvulsiometer (Orchid Scientific India) along with ear electrodes. Video recording was done for all animals. The recordings were later evaluated by a rater who was blinded to the treatment given. Parameters like total time of tonic flexion, tonic extension, and clonic phase duration were calculated by stopwatch. The endpoint for anticonvulsant activity in MES is the inhibition of tonic hind limb extension.

PTZ Induced Seizure: Animals were divided into six groups, each group having eight animals. Different groups received different treatments administered orally for 14 days. Group 7(vehicle control) received Normal saline. Group 8(active control) received Sodium Valproate (300mg/kg). Group 9(low-dose herb) and Group 10(high-dose herb) received *Musa paradisiaca* in the doses of (100 mg/kg) and (200mg/kg) respectively. Group 11(sub-therapeutic active control + high dose herb) was given Sodium Valproate (150mg/kg) + *Musa paradisiaca* (200mg/kg) and Group 12(sub-therapeutic active control+low dose herb) was given Sodium Valproate (150mg/kg) + *Musa paradisiaca* (100mg/kg). On day 14, 60 minutes after the last dose, PTZ was administered intraperitoneally (i.p.) in the dose of 75 mg/kg and animals were placed into an individual plastic cage for observation for 60 minutes and video recording was done for initial 15 minutes. Parameters like time of onset of first myoclonic jerk, time of onset of tonic-clonic seizures, duration of seizures and intensity of seizures by using Racine's seizure intensity scale and % protection against mortality were calculated. Delay in the onset of a first myoclonic jerk, delay in onset or prevention of tonic-clonic convulsion and protection from mortality was taken as the measure of anti epileptic activity.

Statistical Test: Parametric data were analyzed using ANOVA (Analysis of variance) followed by Tukey Kramer post-test and for non-parametric data, Kruskal Wallis followed by Dunn's multiple comparison tests were used. Data are presented as the Mean ± Standard error of the mean (SEM). All statistical analyses were performed using Graph Pad Instat Version 3.06 (Graphpad Software Inc.). A value of $p< 0.05$ was considered statistically significant.

Ethical Approval: The Institutional Animal Ethics Committee (IAEC) of Government Medical College, Bhavnagar, Gujarat, India approved this study (IAEC Approval no.-74/2019). Guidelines of the Committee for Control and Supervision on Experiments on Animals (CCSEA), Ministry of Forest and Environment, Government of India were followed during the conductance of this study.

RESULTS:

MES Test: Statistical analysis of data is done by Kruskal –Wallis test followed by Dunn's multiple comparison test for tonic flexion. For tonic extension data analysis is done by one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. For clonic phase data analysis is done by one way analysis of variance

(ANOVA) followed by Tukey- Kramer multiple comparison test. Total hind limb extension and tonic flexion phase were prevented in Phenytoin 50mg/kg (Group 2), High dose *Musa paradisiaca* 200mg/kg (Group 4) and in add on groups (group 5&6). Low dose *Musa paradisiaca* 100mg/kg failed to prevent total hind limb extension. The clonic phase was present in all the groups. A significant reduction in the duration of clonic phase was seen in both add on groups (group 5&6) as compared to vehicle control (Group 1). Reduction in the duration of clonic phase was significant in group 5 (phenytoin 25mg/kg+ *Musa paradisiaca* 200mg/kg) compared to active control (Group 2- phenytoin 50mg/kg).

TABLE 1 EFFECT OF VEHICLE AND TEST DRUG ON MES INDUCED SEIZURE IN *SWISS* ALBINO MICE (n=8)

Treatmentgroups	Dose (Oral)	Tonic flexion(secs) (mean± SEM)	Tonic extension(secs) (mean± SEM)	Clonic phase (secs) (mean±SEM)
Group- 1Vehicle control	2.5ml/kg	1.60±0.1254	14.25±0.8964	11.81±0.6856
Group- 2 Phenytoin	50mg/kg	Prevented	Prevented	8.93±0.8103
Group- 3 *Musa paradisiaca (Low dose)*	100 mg/kg	1.31±0.1393	10.56±0.8422*	9.90±0.8005
Group- 4 *Musa paradisiaca (High dose)*	200 mg/kg	Prevented	Prevented	9.00±0.9560
Group-5 Phenytoin (25mg/kg) + High dose *Musa paradisiaca*	25mg/kg+200mg/kg	Prevented	Prevented	4.18±0.2214*#
Group-6 Phenytoin (25mg/kg) + low dose *Musa paradisiaca*	25mg/kg+100mg/kg	Prevented	Prevented	6.06±0.2968*

* means p< 0.05 statistically significant as compared to vehicle control.# means p< 0.05 statistically significant as compared to active control.

PTZ Test: Statistical analysis of data is done by one way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test for all variables. % protection is calculated as survived/used multiplied by 100. Myoclonic jerks were produced in all the groups. As compared to vehicle control (group 7) significant increase in duration of onset of 1st myoclonic jerk was seen in Sodium Valproate 300mg/kg (group 8) and add on groups of sub-therapeutic dose of Sodium valproate 150mg/kg + high dose *Musa paradisiaca* 200mg/kg (group 11) and sub-therapeutic dose of Sodium valproate 150mg/kg + low dose *Musa paradisiaca* 100mg/kg (group 12). None of the *Musa paradisiaca* or add on group significantly increased duration of onset of 1st myoclonic jerk when compared to active control (group 8). Seizures were not observed after treatment in active control (group 8-Sodium valproate 300 mg/kg) group as well as sub-therapeutic sodium valproate 150mg/kg + high dose *Musa paradisiaca* 200 mg/kg (group 11). There was an increase in latency of onset and decrease in duration of a seizure in low and high *Musa paradisiacal* (group 9 &10) as well as in sub-therapeutic sodium valproate 150mg/kg + low dose *Musa paradisiaca* 100 mg/kg (group 12) as compared to vehicle control (group 7).

TABLE 2: EFFECT OF VEHICLE AND TEST DRUG ON PENTYLENETETRAZOLE INDUCED SEIZURE IN SWISS ALBINO MICE (n=8)

Treatment groups	Dose (Oral)	1st myoclonic jerk (secs) (mean± SEM)	Latency of onsetof seizures (secs)(mean± SEM)	Duration of seizures (secs)mean± SEM	% Protection	Seizure intensity stage mean±SEM
Group- 7 (Vehicle control)	2.5ml/kg	42.88±3.35#	55±4.76#	20.25±1.40#	0%	6±00#
Group- 8 Sodium Valproate	300 mg/kg	256±23.25*	Prevented	Prevented	100%	1.25±0.16*
Group- 9 *Musa paradisiaca (Low dose)*	100 mg/kg	63.38±5.44#	113.63±9.73*#	13±1.31*#	0%	6±00#
Group- 10 *Musa paradisiaca (High dose)*	200 mg/kg	92±10.19#	167±18.81*#	7.5±0.76*#	100%	4.25±0.16*#
Group-11 Sodium Valproate (150mg/kg) + High dose *Musa paradisiaca*	150 mg/kg +200 mg/kg	267.25±15.12*	Prevented	Prevented	100%	1.13±0.125*

Group-12 Sodium Valproate(150mg/kg) + Low dose *Musa paradisiaca*	150 mg/kg +100 mg/kg	127.63±20.33*#	222.38±21.24*#	7.125±0.92*#	100%	4±00*#

* means p< 0.05 statistically significant as compared to vehicle control. # means p< 0.05 statistically significant as compared to active control.

DISCUSSION: Maximal electroshock (MES) seizures and subcutaneous pentylenetetrazole (PTZ) test are two models that have been used for several decades and still remain the mainstay as key preclinical tests for random screening aimed for the detection of new anti-epileptic drugs working on generalized tonic-clonic seizure and absence seizure, respectively.

Maximal electroshock seizure test helps the testing of drugs acting on Na^+ channel or Ca^{2+} channel, but majority of standard and newly developed anticonvulsant drugs acting through another mechanism like GABA enhancement or glutamate antagonism are also effective in the MES model [13, 14]. In the present study, phenytoin and *Musa paradisiaca* at high dose (200mg/kg) abolished total hind limb extension as well as tonic flexion phase but failed to do that in a low dose (100mg/kg). Phenytoin, as well as *Musa paradisiaca*, failed to prevent clonic phase but there was significant reduction in duration of the clonic phase in both the add-on groups as compared to vehicle control. This prevention of tonic extension and flexion in the MES test by the high dose of *Musa paradisiaca* may be due to the presence of flavonoids which have got anticonvulsant and CNS depressant activity [15]. Flavonoids exert antiepileptic activity through modulating $GABA_A$-Cl^- channel complex because of their structural similarities with benzodiazepines [14].

PTZ is a tetrazole that acts by antagonizing the inhibitory GABAnergic neurotransmission by blocking its chloride channel, activating glutamate receptors (NMDA) and producing oxidative stress to neuronal cells [14, 16]. During this test following I.P. pentylenetetrazole mice follow stereotype response following Racine scale stages and progress to myoclonic jerk and tonic-clonic seizure [17]. In the present study, myoclonic jerks were produced in all the groups. However, a significant increase in the duration of onset of 1st myoclonic jerk was seen in Sodium Valproate and add-on groups as compared to vehicle control.

A significant increase in the duration of latency of onset of seizures and decrease in seizure duration was seen in phenytoin, high dose (200mg/kg) and low dose (100mg/kg) *Musa paradisiaca* and both add-on groups as compared to vehicle control. Seizure intensity staging for PTZ induced seizures i.e. for behavioural patterns during a seizure showed a significant difference in intensity stage of high dose *Musa paradisiaca* and add-on groups as compared to vehicle control, but not in low dose *Musa paradisiaca* (100 mg/kg). *Musa paradisiaca* contains flavonoids that act on $GABA_A$ channels and also have antioxidant activity[7, 9]. This might be the reason for its anticonvulsant mechanism in PTZ induced seizure.

CONCLUSION: The result obtained from this study shows that for MES model *Musa paradisiaca* in high dose prevented convulsions. A combination of high dose and low dose *Musa Paradisiaca* + sub-therapeutic phenytoin produced an effect similar to the therapeutic dose phenytoin. Thus a high dose of *Musa paradisiaca* can be used alone or high / low dose of *Musa Paradisiaca* with sub-therapeutic dose of phenytoin for generalized tonic-clonic seizure. In PTZ model, high dose *Musa paradisiaca* in combination with sub-therapeutic sodium valproate showed result similar to sodium valproate which suggests a synergistic role of the herb with sodium valproate in the prevention of absence seizures. This will allow the use of combination therapy which will reduce adverse effects associated with a therapeutic dose of sodium valproate.

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