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ANTICONVULSANT ACTIVITY OF BORASSUS FLABELLIFER LEAF EXTRACTS

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ABSTRACT

To determine the anticonvulsant activity of leaf extract of Borassus flabellifer Linn. in Wistar albino rats and in order to verify the traditional use of the plant in the treatment of epilepsy. The Maximal Electro Shock Seizure (MES) and Pentylenetetrazole (PTZ) were used for assessing the anticonvulsant activity of alcoholic and aqueous leaf extracts of Borassus flabellifer on wistar albino rats. The acute toxicity study was conducted as per guidelines set by OECD, and no adverse effects or mortality were detected up to 4g/kg,p.o.,based on the results obtained from the study the dose for anticonvulsant activity was fixed to be 100mg,200mg,400mg/kg b.w.,for dose dependent study. The alcoholic extract (400mg) of Borassus flabellifer had shown potent anticonvulsant activity than other extracts when compare to control group and the phenytoin and diazepam treated animals shown 100 percent protection against convulsant.The result from the study indicate that the 400mg of alcoholic leaf extract of Borassus flabellifer Linn. may be beneficial for anticonvulsant activity.

KEYWORDS: Borassus flabellifer, Anticonvulsant activity, MES, PTZ.

1. INTRODUCTION

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences of this condition.[1] The goal of anti-epileptic drug (AED) therapy is to achieve complete seizure control with a single drug taken once or twice a day without side effects. Approximately 70-80% of patients who develop epilepsy may expect to have their seizures controlled with a single drug, while others need combination therapy for improved seizure control. Limitations with conventional AEDs highlighted the need for developing newer agents for epilepsies.[2] The currently used antiepileptic drugs fail to provide satisfactory seizure control for nearly 15-20% patients with epilepsy especially those of partial epilepsies. For such patients combinations of antiepileptic drugs are often prescribed in attempts to improve seizure control. However, toxicities associated with these drugs can further compromise quality of life while drug interactions may complicate clinical management.[3] *Borassus flabellifer* L. belongs to the family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated and naturalized throughout India. The different parts of *Borassus flabellfer* are being used for medicinal properties.

In the previous study, several steroidal saponins , poly saccharides and triterpenes were isolated from the fruit pulp and seeds, young shoots of *Borassus flabellifer* contains gum, albuminoids, fats and the fresh pulp contains vitamin A ,B and C, the inflorescence contains borassoside, dioscin and also contains sucrose, bitter compound flabelliferins.[4]

Plant yields a black gum, a good source of Vitamin B complex, extract in stroke, head ache, earache, epilepsy, scabies, syphilis, ulcers, vomiting. Bark used as dentifrices, flowers in uterus tumors. Fruits as tonic for asthmatic patients, gonorrhea and given in gas troubles. Roots used as cooling medicine and restorative, diuretic and anthelmintic, in gastritis.[5]

However the anticonvulsant activities of *Borassus flabellifer* on wistar rats have not been documented. Hence the present study is undertaken for the phytochemical investigation of and *Borassus flabellifer* to evaluate their traditionally claimed antiepileptic activity in wistar albino rats.

2. MATERIALS AND METHODS

2.1Plant material and extraction

Borassus flabellifer leaves were collected from Perambalur district in Tamilnadu and authenticated by Dr.(Prof)V.Sathyanathan Thirumala college of Pharmacy, Nizamabad, AP.The shade-dried leaves of *Borassus flabellifer Linn*, family Arecaceae were reduced to fine powder (# 40 size mesh) and around 300

gms of powder was subjected to successive hot continuous extraction (Soxhlet) with petroleum ether, and alcohol. Finally the drug was macerated with chloroform-water. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50^0C. After the effective extraction, the solvent were distilled off, the extract was then concentrated on water bath and the extract obtained with each solvents was weighed. Its percentage was calculated in terms of air-dried weight of plant material. The colour and consistency of the extracts was noted. The obtained alcoholic and aqueous extracts were subjected to chemical investigation and pharmacological screening for its anticonvulsant activity by using MES test and PTZ induced seizures.

2.2 Animal Selection

Wistar albino rats of either sex weighing between 150-200gms were selected for experiments. They were employed for assessing antiepileptic activity. The animals were housed in propylene cages and fed on standard laboratory diet and water *ad libitum.* maintained at an ambient temperatures of 25±2ºC and exposing them to 12hlight/dark cycle.The ethical clearance was obtained from Institutional animal Ethical Committee (registration number1798/PO/E/15/CPCSEA) before the experiment.

2.3 Drugs

Pentylenetetrazole (PTZ; Sigma Poole UK), phenytoin sodium (Epsoline injection, Zydus Neurosciences, India) and diazepam (Calmpose injection, Ranbaxy, India) were used after appropriate dilution with distilled water.

2.4 Toxicity assessment

The acute oral toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD) regulations.[6]

The alcoholic and aqueous extracts of Borassus flabellifer were not shown any toxicity. So 1/20, 1/10 and 1/5th of the dose selected for this study.

2.5 Anticonvulsant activity against Maximal Electroshock Seizure (MES)

The experimental animals were divided into eight groups of six rats each Group I (control) received normal saline (1ml / rat, p.o), Group II received standard drug Phenytoin (25 mg / kg, i.p) and Group III received 100 mg / kg, p.o of ethanol extract, Group IV received 200 mg / kg, p.o of ethanol extract, Group V received 400 mg / kg, p.o of ethanol extract, Group VI received 100 mg / kg, p.o of aqueous extract, Group VII received 200 mg / kg, p.o of aqueous extract, Group VII received 400 mg / kg, p.o of aqueous extract, 1 h prior to the induction of convulsions respectively. Maximal electroshock of 150 mA current for 0.2 seconds was administered through ear electrodes to induce convulsion in all the experimental animals.[7] The severity of convulsions was evaluated by measuring (sec) the duration of tonic flexion, tonic extensor, clonus, stupor and recovery phase in all the grouped animals and compared with standard.

2.6 Anticonvulsant activity against PTZ induced seizures

The animals were divided into eight groups of six animals each. Group I (control) received normal saline (1ml/rat, p.o), Group II received standard drug diazepam (4 mg/kg, i.p) and Group III received ethanol extract (100 mg/ kg, p.o.), Group IV received ethanol extract (200 mg/ kg, p.o.), Group V received ethanol extract (400 mg/ kg, p.o.), Group VI received aqueous extract (100 mg/ kg, p.o.), Group VII received aqueous extract (200 mg/ kg, p.o.), Group VIII received aqueous extract (400 mg/ kg, p.o.). Pentylenetetrazole (80 mg/kg) was administered intraperitoneally to induce convulsions to all the grouped animals at 1hr post treatment of saline (vehicle), standard drug, ethanol extract and aqueous extract.[8] The anticonvulsant effect was assessed by measuring the time in sec for the test drugs to delay the onset of action/protection against PTZ (chemo shock) induced convulsions and mortality time was recorded.

2.7 Statistical analysis

All the results were expressed as Mean ± SEM.The statistical significance was analyzed by performing one way ANOVA followed by Dunnett's *t*-test. $P < 0.01$ implies significance.[9]

3. RESULTS AND DISCUSSION

All extracts of *Borassus flabellifer* were subjected to assessment of 1) Acute toxicity study:2) Anticonvulsant activity by using MES & PTZ induced convulsion in albimo rats.No toxic effects produced from *Borassus flabellifer* extracts. So I selected minimum, medium and high dose(1/5 th(100mg), 1/10 th(200mg), 1/20 th (400mg) of the maximum toxicity dose 2000mg/kg bow. The above dose was taken for subsequent anticonvulsant activity.

The model used to evaluate the effectiveness of various extracts of *Borassus flabellifer* leaves were maximal electroshock seizure test and pentylene tetrazole seizure test.

The MES model is generally used to evaluate the anticonvulsant drugs against generalized tonic clonic seizure (grandmal) in rodents which is related to intensity of current stimulus and dose. MES produced various phases of convulsion i.e. flexion, extension, clonus and stupor. The duration of tonic extension of the hind limb was used as end point preservation or decrease in the duration of hind limb extension was considered as a protective action.

The various extracts of *Borassus flabellfer* were given orally, the result of all the extracts is compared with the result produced by control.

The data resulted from anticonvulsant effect of different extracts showed that the 400mg of alcoholic extract of *Borassus flabellifer* decreased the duration of flexion by(0.5±0.22sec) which is most significant ($p<0.01$) when compared to control (3.33±0.33 sec) and the effect produced by alcoholic extract 200mg (0.5±0.22 sec),Alcoholic extract 100mg (0.83±0.47sec),aqueous extract 400mg(1.5±0.34sec),aqueous extract 200mg(1.83±0.16 sec) and aqueous extract 100mg (4.83±0.60 sec) of *Borassus flabellifer* extracts in MES model.

The alcoholic extract 400mg decreases the flexion (0.5±0.22), extension (10.16±0.47), clonus (7.66±0.49) and stupor (66.66±6.82) sec. When compared to control flexion(3.3±0.33), extension (11.5±0.67), clonus (11.5±1.17), stupor(104±4.82). In other words the alcoholic extract 400mg decreased the duration of hind limb extension, clonus and also the duration of stupor phase which indicates, it possesses potent anticonvulsant activity against generalized tonic, clonic seizure (grand mal). Other extracts viz alcoholic extract 100mg, alcoholic extract 200mg, aqueous extract100mg, aqueous extract 200mg and aqueous extract 400mg does not showed statistically significant effect in extensions or phase as compared to control. The standard drug phenytoin in a dose of 25mg/kg h.w. provided 100% protection and also significantly reduced the duration of stupor (19.16±0.94 sec) when compared to control (104±4.82sec).

80mg/kg sc. was used for inducing convulsions in all eight groups, alcoholic group 400mg, onset of time (seconds) to show convulsions such as jerks, clonus and extensor were (54.16±1.44), (95.83±1.74), (107.16±4.19) as compared to control group (48.16±2.27), (83.16±1.38), (92.5±1.76) respectively. The animals in alcoholic extract400mg treated group shown a significant difference in delaying the onset of convulsions.

In MES induced seizures the Standard drug Phenytoin (25 mg/kg, i.p.) reduces the hind limb tonic extension by inhibiting voltage dependent Na+ channels. On the other hand, Diazepam (4 mg/kg, i.p.) prevents the convulsions induced by PTZ by enhancing gamma amino butyric acid type A (GABAA) receptor mediated inhibitory neurotransmission.[10-12]

Table No.1 Effect of *Borassus flabellifer* Extract against MES Induced convulsions

Drug	Dose mg/Kg b.w.	Route of Administration	Time (Sec) in various phases of convulsions (Mean±SEM)				
			Flexion	***Extension***	***Clonus***	***Stupor***	***Recovery***
Control (Saline)	1ml/rate	Oral	3.33±0.33	11.5±0.67	11.5±1.17	104±4.82	Mortality
Standard phenytoin	25	Intra peritoneal (i.p)	1.5±0.22**	0.00±0.00**	2.5±0.56**	13.16±0.94**	Recovery
Alcoholic Extract	100	Oral	0.83±0.47**	11.83±0.87	10.5±0.56	84.83±19.53	Recovery
Alcoholic Extract	200	Oral	0.5±0.22**	9.66±0.66	6.0±0.63**	80.0±4.25	Recovery
Alcoholic Extract	400	Oral	0.5±0.22**	10.16±0.47	7.66±0.49**	66.66±6.82*	Recovery
Aqueous Extract	100	Oral	4.83±0.60*	11±0.57	8.83±0.47*	87.16±2.56	Recovery
Aqueous Extract	200	Oral	1.83±0.16*	11±0.57	7.83±0.47**	76.33±8.3	Recovery
Aqueous Extract	400	Oral	1.5±0.34**	12.16±0.79	7.66±0.49**	66.16±6.26*	Recovery

One way Anova followed by Dunnet's 't' test
Note: n=6 in each group. *P<0.05, **P<0.01,

Table No:2 Effect of *Borassus flabellifer* Extract against PTZ Induced convulsions

Sl.No.	Drug	Dose	Route of Ad.	Times (sec) in various phases of convulsions			Recovery/ Mortality
				Jerks	Clonus	Extensor	
1.	Control Saline	1ml/rat	Oral	48.16±2.27	83.16±1.38	92.5±1.76	Mortality
2.	Standard Diazepam	4 mg	IP	0.00±0.00**	0.00±0.00**	0.00±0.00**	Recovery
3.	Alcoholic extract	100 mg	Oral	51±1.12	78±5.84	92.83±3.60	Recovery
4.	Alcoholic extract	200 mg	Oral	53.16±0.82	84.66±1.74	103.66±2.97*	Recovery
5.	Alcoholic extract	400 mg	Oral	54.16±1.44*	95.83±1.74**	107.16±4.19**	Recovery
6.	Aqueous extract	100 mg	Oral	52±1.06	74±0.93	88.66±2.89	Recovery
7	Aqueous extract	200mg	Oral	49.33±1.94	81±1.63	86±3.32	Recovery
8	Aqueous extract	400mg	Oral	52.66±0.61	89.33±1.54	106.33±2.94*	Recovery

One way Anova followed by Dunnet's 't' test
Note: n=6 in each group. *P<0.05, **P<0.01

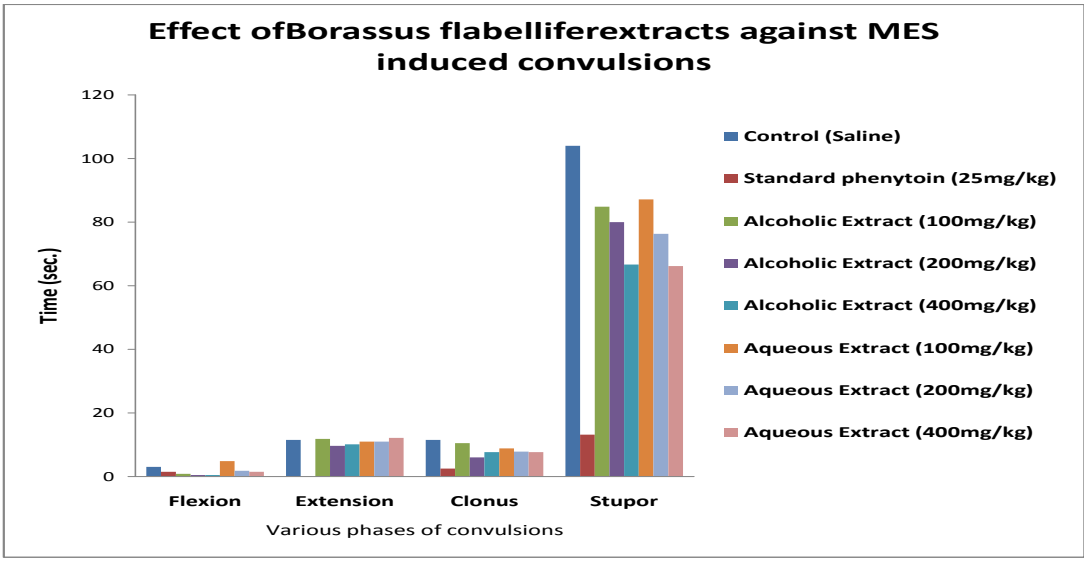

Figure1: Effect of Borassus flabellifer leaf extracts against MES induced convulsions

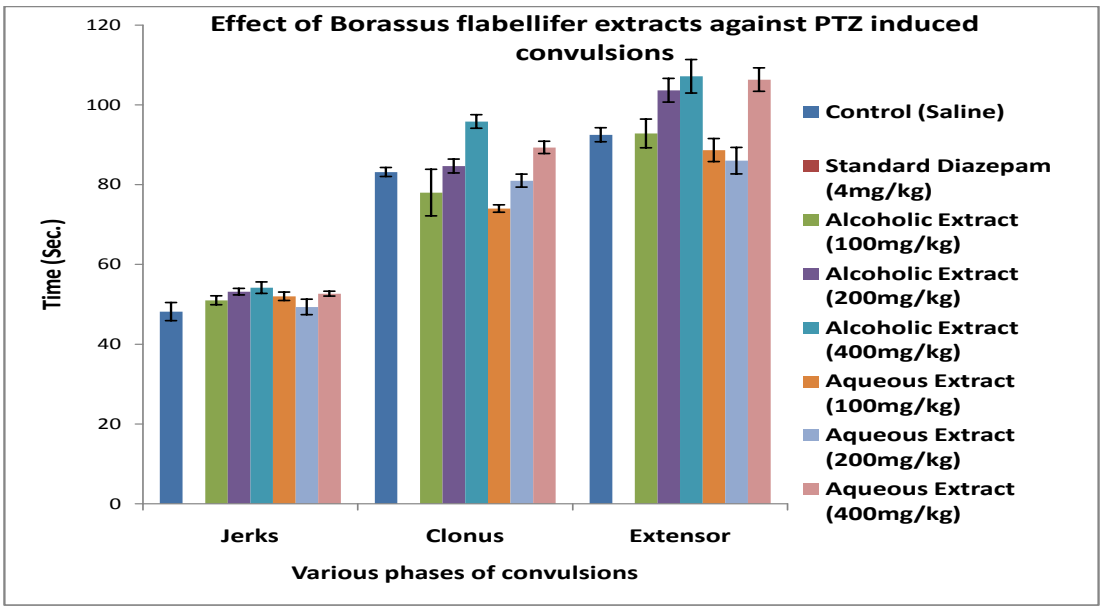

Figure2: Effect of Borassus flabellifer leaf extracts against PTZ induced convulsions

4. CONCLUSION

The ethanol extract 400mg of *Borassus flabellifer* leaves has exhibited significant anticonvulsant activity against both MES and PTZ induced convulsions revealing the multiple mechanism of action which could, possibly, be due to inhibition of voltage dependent Na+ channels by blocking glutaminergic excitation mediated by N-Methyl- D-aspartate (NMDA) receptor, by reducing ca2+ channels or by enhancing gamma amino butyric acid type A ($GABA_A$) receptors mediated Phytochemical investigation of ethanol extract revealed the presence of flavaonoids, tannins triterpenes and carbohydrates. The flavonoids are known to possess action on central nervous system. Hence, the presence of flavonoids and other phenolic compounds in ethanolic extract could be attributed for the observed significant anticonvulsant activity.

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