Original Article

Experimental Evaluation of Anti Convulsant Property of Myristica Fragrans

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ABSTRACT

Background: Myristica fragrans (Nutmeg) has been traditionally known since ancient days for its nutritive and various effects in the body. In traditional medicine, Myristica had been used for its digestant, carminative and anti-ulcer property, as an analgesic, anti-inflammatory, anti-convulsant and many more.

Aim: The aim of the present study is evaluation of anticonvulsant property of extract of Myristica fragrans in albino rats.

Materials and Methods: Five groups consisting of six rats each were taken, Gr1 served as control, Gr 2 as standard, given phenobarbitone, Gr 3, 4 and 5 were test groups with various doses of Myristica extract. The convulsions were induced by Pentylenetetrazole. The effects anticonvulsant effects of Myristica are compared with standard anticonvulsant Phenobarbitone.

Result: Myristica fragrans extract showed statistically significant anticonvulsant property in test group.

Conclusion: Myristica fragrans extract has to be tested with other species and electrically induced convulsions also.

Keywords: Myristica fragrans, pentylenetetrazole, phenobarbitone.

INTRODUCTION

Epilepsy is the second most common disorder of the CNS after stroke, affecting about 1% of the population world wide.[1] Even with the best management, optimum seizure control cannot be guaranteed [2], and the disease remains disabling and stigmatizing. Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. 'Seizures' refers to transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons.[3] Causes of seizures are many from infection to neoplasm, head injury and underlying toxic or metabolic disorder. The currently used Anti epileptic drugs fail to provide satisfactory seizure control for nearly 15% - 20% patients with epilepsy. Thus, there is an ever increasing need for newer molecules for treating epileptic seizure. Indeed low concentration of GABA in the brain may be associated with poorly controlled epilepsy[4], so drugs are now being developed in this line.

None of the available medicines have the properties of an ideal drug and hence the continuing search for the ideal drug or drug combination. The present study is to

experimentally evaluate the anti-convulsant property of the myristica fragrans, in chemically induced seizure in rats.

Majority of the drugs are derived from plant products and chemical structures are analysed and the synthetic substances were produced. The fruit of myristica fragrans is commonly called as 'nutmeg' in local language. Nutmegs were purchased from a reliable and authentic shop of market and identified on pharmacognostic basis and was confirmed as Nutmeg by the Botanist and Research Chemist of Central Research Institute of Unani Medicine (CRIUM), Hyderabad.

MATERIALS AND METHODS

Male albino rats weighing 150-200 gms were selected, to avoid the possibility of variation in response with changing hormonal status due to estrous cycle of female on brain exatability, and female rats are known to eliminate several anti epileptic drugs less rapidly than males do.[5]

Pentylene tetrazole (PTZ) is one of the most commonly employed substance for anti-convulsant screening method. The dose 65mg/kg body weight has been found to produce generalized clonic seizures in 100% animals with least mortality. 100 mg of PTZ weighed and dissolved in 5 ml of distilled water (i.e. 20 mg/ml).

The dose calculated for each rat and administered subcutaneously. Distilled water 2 ml is given subcutaneously in the control group

Myristica fragrans extract obtained by using Soxhlet's apparatus[7] and extraction done using petroleum ether of 60-800 C AR grade as solvent. Extract was kept in an incubator at 450C for evaporation of petroleum ether. The resultant residue / extract of nutmeg is almost colourless to pale yellow liquid with characteristic odour. Solubility was found better in Tween 80. The dose is 4-5 gm to produce desired effect. [6]

Phenobarbitone is effective organic anti-seizure agent. It inhibits seizures evoked by PTZ in the dose of 30mg/kg subcutaneously after dissolved in distilled water.

The study protocol was presented to the Institutional Animal Ethics Committee, and was approved, to proceed with the conduction of the experiment.

Method

Prior to the experiment, Acute Toxicity Tests were done; Seven groups of rats, of two each are taken. The petroleum ether extract of Myristica fragrans was administered in doses of 10, 30, 100, 300, 1000, 3000 and 10,000 mg/kg body weight, subcutaneously and are watched for toxic effects and mortality. No mortality was observed in any animal groups after 24 hours. However they exhibited signs of CNS depression with diminished alertness, decreased spontaneous motor activity, grouping of animals closely and decreased touch response. The intensity of those effects started waning after 2 to 3 hours. In all, the animals tolerated the drug well.

The experiment was done employing chemical method to induce seizures with PTZ and for comparing anti-convulsant activity of extract of myristica fragrans, with known standard anti convulsant phenobarbitone. The study was carried out according to the method of Swinyard, Brown and Goodman (1952). [8]

Albino rats were selected for this study because various types of seizures can be produced in them. The experiment was done to employing chemical method to induce seizures with pentylene tetrazole. The dose of pentylene tetrazole varies from 60 mg/kg [5] to 112 mg/kg.[9]

But with a dosage of 65 mg/kg developed generalized clonic convulsions with no/least mortality (LD¬¬¬50 of PTZ was 85 mg/kg).

The animals had free access to food and water prior to the experiment, and the experiments were carried out during 9.00 – 13.00 hrs (to decrease the variability in epileptic responsiveness from time to time in a day).

Albino rats were divided in to 3 groups of 12 each – control (C), standard (S) and Test (T). The control group (C) received 2 ml per rat distilled water subcutaneously over scruff of neck.

The standard group (S) received phenobarbitone 30mg/kg weight dissolved in distilled water and administered subcutaneously over scruff of neck.

The Test group (T) received extract of myristica fragrans in the dose of 5000mg/kg body weight administered subcutaneously over scruff of neck (large doses 5-10 ml of the drug could also be given).[10]

One hour after administration control, standard and test drugs the PTZ in the dose of 65mg/kg was given subcutaneously in right lower quadrant of abdomen. Immediately the stop watch was started, the time taken for onset of seizure, the duration of seizure and time taken for complete recovery were noted. Any other effects, abnormal movements were also noted.

Ethical approval

The protocol was submitted to the Institutional Animal Ethics Committee, and approved by the Committee. The guidelines of CPCSEA were adhered to during the study.

Table 1: Pre-treated with distilled water control 'C' Group N=12

S.No.	Body Wt. of rats in grams	Distilled Water Dose/rat	PTZ mg/rat	CONVULSIONS		
				Time of onset in seconds	Duration In Seconds	Time to recover after seizures in seconds
1	177	2 ml	11.50	410	30	960
2	175	2 ml	11.50	496	38	1210
3	145	2 ml	9.42	445	20	816
4	159	2 ml	10.33	552	26	830
5	223	2 ml	14.49	543	17	940
6	188	2 ml	12.22	384	32	972
7	164	2 ml	10.66	480	30	1140
8	186	2 ml	12.09	396	29	1107
9	190	2 ml	12.35	436	32	958
10	151	2 ml	9.81	518	25	886
11	210	2 ml	13.36	481	26	1204
12	178	2 ml	11.57	392	31	974
Mean	178.83		11.62	461.08	28	999.75
±SD	22.8		1.49	59.27	5.66	135.2
±SE	6.59		0.42	17.11	17.11	39.02

Table 2: Pre-treated with phenobarbitone 30mg/kg. body weight standard Group 's' n=12

S.No.	Body Wt. of rats in grams	Phenobarbitone mg/rat	PTZ mg/rat	CONVULSIONS		
				Time of onset in seconds	Duration In Seconds	Time to recover after seizures in seconds
1	223	6.69	14.49	No convulsions noted		
2	145	4.35	9.42	"		
3	175	5.25	11.37	"		
4	188	5.64	12.22	"		
5	159	4.77	10.33	"		
6	177	5.31	11.50	"		
7	168	5.04	10.92	"		
8	156	4.68	10.14	"		
9	180	5.4	11.70	"		
10	162	4.86	10.53	"		
11	194	5.82	12.61	"		
12	175	5.25	11.37	"		
Mean	175.16	5.25	11.38			
±SD	20.44	0.61	1.33			
±SE	5.9	0.18	0.38			

SD - Standard Deviation
SE - Standard Error

Table 3: Pre-treated with myristica fragrans 5000mg/kg. body weight test group –t n=12

S.No.	Body Wt. of rats in grams	Myristica Fragrans Dose/rat	PTZ mg/rat	CONVULSIONS		
				Time of onset in seconds	Duration In Seconds	Time to recover after seizures in seconds
1	170	850	11.05		No convulsions	
2	176	880	11.44		No convulsions	
3	152	760	9.88	1760	18	832
4	194	970	12.61		No convulsions	
5	166	830	10.79		No convulsions	
6	182	910	11.83	1816	No convulsions	
7	160	800	10.40		17	805
8	175	860	11.18	1255	No convulsions	
9	151	760	9.88		20	846
10	180	900	11.70		No convulsions	
11	158	790	10.27		No convulsions	
12	188	940	12.22		No convulsions	
Mean	171.08	854.16	11.10	1610.33	18.33	827.65
±SD	13.81	68.82	0.89			
±SE	3.98	19.87	0.26			

Table 4: Effect of drugs on PTZ induced seizures in rats

Drug	PTZ induced convulsions			
	Time of onset in seconds	Duration in seconds	Time to recover in seconds	% protected
Control 'C' Distilled water 2ml/rat	461.08+ 59.27	28.0+5.66	999.8 + 135.2	0%
Standard group 'S' phenobarbitone 30mg/kg	-	-	-	100%
Test group 'T' M.fragrans 5000mg/kg	1610.33	18.33	827.66	**75%

- *Results are presented as mean value + S.D.*
- *n=12 in each group.*

*** P<0.001, highly significant, when 'T' was compared with 'C'.*

STATISTICAL ANALYSIS

All the values expressed as mean +SD and the percentage of inhibition were calculated. The test of significance was done by using chi-square test.

RESULTS

There was 100% protection in standard group, 75% protection in test group and zero protection in control group.

DISCUSSION

All animals developed clonic convulsions with PTZ after a mean time of 461.08 +59.27 seconds. Mean duration was 28+5.66 seconds, mean time to recover was 999.75+135.2

seconds (It was also noted that three animals developed a second episode of clonic convulsions).

In standard group pretreated with phenobarbitone, no animal developed clonic convulsions for one hour after PTZ administration. 100% protection against seizure in this group.

In test group 9 out of 12 rats (75%) were completely protected from PTZ induced seizure. Three animals developed clonic convulsion after a mean time of 1610.33 seconds, mean duration of convulsions was 18.33 seconds, mean time to recover after seizure was 827.66 seconds. No second episode of convulsions.

The test was compared with control group, it was found to be highly significant i.e. $p<0.001$. There was no statistical significance i.e. $p>0.05$ when test group was compared with standard group.

CONCLUSION

Extract of myristica fragrans has anti convulsant activity, which needs further evaluation in different species and electrically induced convulsions also.

CONFLICT OF INTEREST :

The authors declared no conflict of interest.

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