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Research Article

ANTICONVULSANT ACTIVITY OF *KIGELIA PINNATA* BARK EXTRACT

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ABSTRACT

The present studies reveal that the anticonvulsant activity by *Kigelia pinnata* bark (PTZ) and MES induced convulsions in wistar rats using *Kigelia pinnata* bark extract methanolic (*KPM*) and aqueous (*KPA*) extracts extracted successively. The extracts (aqueous & methanol) contain alkaloids, glycosides, carbohydrates, steroids, tannins, phenolic compounds, proteins, amino acids, saponins and flavonoids. 250 mg/kg and 500mg/kg of *KPM* and *KPA* were given intraperitonially. The latency of seizures, death time and % of mortality were observed. *KPM* gave significant protection against the PTZ (pentylene tetrazole) and MES (maximal electro shock) induced convulsions. The p value in PTZ is p< 0.0001and MES, p< 0.0001and p< 0.0001.

Keywords: *Kigelia pinnata*, Anticonvulsant, PTZ, MES.

INTRODUCTION

Kigelia pinnata (Family: *Bignoniaceae*) also called *Kigelia africana* is a plant that is widely distributed in the south, central, West Africa and India. Locally known as the Cucumber or Sausage tree because of the huge fruits (average 0.6 m in length and 4 kg in weight), which hangs from long fibrous stalks but do not splits easily[1-2]. The tree can grow to more than 20 m tall. It is found mostly in riverine areas. Its distribution is restricted to the wetter areas. Different parts of this plant have been claimed to serve various purposes in different parts of the world [3].The fruits pods are very fibrous with numerous seeds and tend to be inedible to humans as well as being poisonous when unripe. However in Malawi during famine, the seeds are roasted and eaten. The Tonga applies powdered fruit as a dressing to wound. Unripe fruit is used in central Africa as a dressing for wounds, and in the treatment of haemorrhage and rheumatism[4]. Venereal diseases are commonly treated with the extracts of *Kigelia pinnata* usually in palm wine as oral medication. The fruits and barks, ground and boiled in water are also taken orally or used in treating stomach ailments. The Shona people tend to use the bark as powder or infusion for application to ulcers, or applied in treatment of pneumonia[5].

Most commonly, traditional healers have used the sausage tree to treat a wide range of skin ailments from relatively mild complaints such as fungal infections, boils, psoriasis and eczema, through to the more serious disease like leprosy, syphilis and skin cancer. Previous studies of the fruits of *Kigelia pinnata* showed some anti-bacterial activity[6]. However there is no report on the, anticonvulsant properties of the bark of this plant. This report therefore presents studies on the anticonvulsant properties of the ethanolic and aqueous extract of the bark.

MATERIALS AND METHODS

Collection and authentication of plant material

The *bark* was collected in February 2010 from forest Department of Etawah, U.P., India & authenticated by Dr.Harish K.Sharma, Ayurvedic Medical College, Devangere; Karnataka, India, bark was collected & dried under shade. A voucher specimen No.AU 100 was deposited in the herbarium of the institute.

Extraction

Methanolic extract: 500 g of the powdered material was mixed with absolute alcohol (2.5 ltr) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, the extract were passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 3.78%, which was stored in universal bottles and refrigerated at 4°C prior to use.

Aqueous extract: 500 g of the powdered material was mixed with distilled water (2.5 l) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, the extract were passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 5.93%, which was stored in universal bottles and refrigerated at 4°C prior to use.

Phytochemical screening: Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the bark powdered using standard procedures[7].

Animals: Male wistar rats weighing 200-300 gm of either sex were procured from animal house of the Sir Madanlal Institute of Pharmacy (approved by the animal Ethical committee).All the animals are kept in standard polypropylene cages and maintained under standard conditions: temperature (24 ± 10 C), relative humidity (45-55 %) and 12:12 light: dark cycle. The animals were fed with standard rat pallet and water. The animals were allowed to acclimatize to laboratory conditions 48 hrs before the start of the experiment. Groups of 6 rats (200-300 gm.) were used in all sets of experiments. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC).

PTZ to study the anticonvulsant activity

Animals in Group I served as control were, treated with vehicle (4 % acacia) orally. Group II served as standard received diazepam (5 mg/kg i.p.). Group III and Group IV received *KPM* at the dose levels of 250 mg/kg and 500 mg/kg i.p. respectively. Group V and Group VI received *KPA* at the dose levels of 250mg/kg and 500 mg/kg i.p.., respectively. One hour after administration of vehicle, standard drug, *KPM* and *KPA* to the respective groups, the animals were treated with PTZ (Pentylene tetrazole, 80 mg/kg) subcutaneously[8]. Each animal was placed in to individual polypropylene cage and were observed initially for 30 min and later up to 24 hrs. The following parameters were recorded during test session of initial 30 min and up to 24 hrs: Latency (onset of clonus), Onset of tonic convulsions, and Status of animal after 30 min, Status of animal after 24 hrs and Percentage protection[9].

MES induced convulsions

MES seizures were induced by Electroconvulsiometer. Maximal seizures were elicited by 60 Hz alternating current of 150 mA intensity for 0.2 sec using corneal electrodes. A drop of electrolyte solution 0.9% sodium chloride with lignocaine was applied to the corneal electrodes, which ensures better contact and the mortality rate to zero[10-11]. This current intensity elicited complete tonic extension of the hind limbs in control rats. For recording various parameters, rats were placed in a clear rectangular polypropylene cage with an open top, permitting full view of the animal motor

responses to seizure the pilot study of various phases of convulsions, like tonic flexion, extension, stupor and mortality due to convulsions were observed. Here phenytoin 25 mg/kg was used as standard instead of diazepam[12].

Statistical analysis

Values were expressed as mean ± SEM from 6 animals. Statistical differences in mean were calculated using one way ANOVA followed by Dunnett's test. p< 0.0001 were p< 0.0001 were considered Statistical.

RESULTS

Preliminary hytochemical studies

The preliminary phytochemical screening of ethanolic and aqueous extract shows the presence of alkaloids, glycosides, carbohydrates, tannins, phenolic compounds, proteins, amino acids, saponins, flavonoids.

PTZ (Pentylene tetrazole) induced convulsions

250 mg/kg and 500mg/kg of *KPM* exhibited a significant anticonvulsant effect by increasing latency, onset of clonic convulsion and decreases onset of tonic seizures.

After 30 min of interval, 67 % and 84 % of animals survived. In *KPA*, after 30 min 50 % and 67 % animals survived at a dose of 250 mg/kg and 500mg/kg. 67 % and 67 % animals survived in *KPM* at dose of 250mg/kg and 500mg/kg after 24 hrs. While 34 % and 50 % survived in 250 mg/kg 500 mg/kg in *KPA* survived as shown in Table 1.

Effect on MES induced convulsion

The poly herbal extract exhibited a dose dependent significant (*P*<0.0001and *P*<0.0001) reduction in various phases of epileptic seizure on comparison with the reference standard phenytoin 25 mg/kg, i.p. There was also a significant reduction in the time required for the righting reflex (recovery) in the extract treated groups (Table 2).

Table 1: Effect of KPM and KPA on PTZ induced convulsions model

Group	Treatment	Latency(onset of colonic convulsion) sec/min	Onset of tonic convulsion sec/min	Status of animal after 30 min		Status of animal after 24 hrs	
				No of alive animals	% protection	No of alive animals	% protection
Group 1	Control(4% acacia)	47.16±0.601	744.33±1.229	0	0	0	0
Group 2	Diazepam (5 mg/kg)	No colonus	No tonic±0.00	All	100	All	100
Group 3	*KPM* (250 mg/kg)	222.83±0.945	574.00±0.764	4	67	4	67
Group 4	*KPM* (500 mg/kg)	236.00±0.683	565.00±2.671	5	84	4	67
Group 5	*KPA* (250 mg/kg)	144.50±1.118	31.83±0.477	3	50	2	34
Group 6	*KPA* (500 mg/kg)	191.50±0.922	614.00±1.211	4	67	3	50

Values expressed are mean SEM from 6 rats. p< 0.0001*** as compared to control group.

Table 2: Effect of KPM and KPA extract on MES induced convulsions in rats

Group	Treatment	Flexion	Extension	Colonus	Stuper	Recovery
Group 1	Control(4% acacia)	8.45±0.177	16.45±0.177	16.70±0.294	9.30±0.198	209.33±2.654
Group 2	phenytoin (25 mg/kg)	4.28±0.095^{a***}	00	9.28±0.095^{a***}	2.18±0.106^{a***}	201.00±1.265
Group 3	*KPM* (250 mg/kg)	3.33±0.088^{b***}	1.43±0.067^{b***}	5.18±0.083^{b***}	22.83±1.249^{b***}	132.00±2.049
Group 4	*KPM* (500 mg/kg)	2.16±0.071^{b***}	1.13±0.049^{b***}	4.41±0.060^{b***}	18.50±0.428^{b***}	114.00±0.632
Group 5	*KPA* (250 mg/kg)	3.517±0.095 ^{b***}	1.95±0.112 ^{b***}	5.63±0.088 ^{b***}	24.33±0.843 ^{b***}	134.50±1.979
Group 6	*KPA* (500 mg/kg)	2.283±0.060 ^{b***}	1.33±0.071 ^{b***}	5.20±0.163 ^{b***}	21.50±0.619 ^{b***}	117.00±0.856

Values represent mean of six observations.
Comparisons between: a – Group I vs. Group II, b – Group II vs. Group III, Group IV, Group V, and Group VI.
Statistical significant test for comparison was done by ANOVA.

DISCUSSION

In Pentylene tetrazole induced seizure test parameters like latency, onset of tonic convulsions, clonic convulsions and percent protection were observed in the test groups (p<0.0001), showing strong antiepileptic effect. The death rate was 100% in Group I. 5 mg/kg of Diazepam, prevents tonic and clonic convulsions and offered 100% protection. 250 mg/kg and 500mg/kg of *KPM* and *KPA* exhibited a significant anti convulsant effect by increasing onset of clonic convulsion and by decreasing onset of tonic seizures. After 30 min of interval 67 % and 84 % of animals survived with a dose of 250 mg/kg and 500 mg/kg of *KPM*. While 50 % survived with the dose of 250 mg/kg and 67 % survived with 500 mg/kg of *KPA*.

After 24 hrs, the % protection of animals was, 67 % and 67 % for 250 mg/kg and 500 mg/kg of *KPM* respectively. 34 % and 50 % protection after 24 hrs in 250 mg/kg 500 mg/kg in *KPA* survived (p value is p< 0.0001 as compared to control). Here, *KPM* shows potent anticonvulsant activity compare to *KPA*. These results further indicates the strong protective effect of test drug against a known

epileptic agent In Maximum Electro Shock induced seizure test, shown anticonvulsant effect by increasing the onset of clonic convulsion time and by decreasing the time of extensor of test groups reduced to significant level as compared to control group ($p<0.0001$ and $p< 0.0001$ as compared to control). These results indicate the strong protective effect of 250 mg/kg and 500 mg/kg of KPM against known epileptic agents. There are some evidences about anticonvulsant effect of this fatty acids[13-14] and some flavonoids[15-16].Therefore it seems that antiseizure effect of *Kigelia pinnata* may be due to part of linoleic acid ,cinnamic acid[17].and/or flavonoid compounds present in the extracts. Thus the results of both doses of KPM, demonstrates a very striking and potent antiepileptic activity, it may be useful in both types of epileptic conditions like Grand mal and Petit mal epilepsy. It demonstrated specific nature of pharmacological effect of *Kigelia pinnata bark.*

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