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EVALUATION OF ANTICONVULSANT ACTIVITY OF COW URINE BETEL VINE EXTRACT IN MICE

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Abstract: The present study was designed to investigate the anticonvulsant activity of Cow urine betel vine extract in rats. Anticonvulsant activity was performed by using the two models Maximal Electric Shock (MES) induced convulsions and Pentylene tetrazole (PTZ) induced convulsions**.** The animals were fed with Cow urine betel vine extract at the dose of 250 and 500mg/kg b.w orally for a period of 14 days. The pretreated extract reduced the convulsions in a dose dependent manner which was determined by taking the duration of flexion, extensor, clonus and stupor phase and Percentage of inhibition of seizures relative to controls was calculated.

Keywords: Anticonvulsant activity, MES, PTZ, Cow urine betel vine extract.

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INTRODUCTION

Epilepsy or convulsion is a neurological disorder in which a person has two or more recurrent unprovoked seizures. Seizure is a paroxysmal event that is due to abnormal, excessive and hypersynchronous discharge from an aggregate of central nervous system neurons. Epilepsy is the second most common disorder of the central nervous system after stroke and up to 5% of world population develops epilepsy in their lifetime [1]. The current therapy of epilepsy with modern anticonvulsive drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current anticonvulsive drugs therapy. Traditional system is believed to be an important source of chemical substances with potential therapeutic effects. Herbs may have antiepileptic effects in several ways. Some herbs may increase brain levels and/or the binding of nerve transmitter gamma aminobutyric acid (GABA), which quiets nerve activity [2,3]. Concurrently, phytochemicals identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics. This has accelerated the global effort to harness and harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in convulsion.

Piper betel is an important medicinal plant, which finds an extensive use in Ayurveda and Unani system of medicine (4). Piper betel is commonly known as betel vine and belongs to the family Piperaceae. The betel vine is reported to have antibacterial, antimicrobial, antidiabetic, anticancer, antioxidant and hepatoprotective and immuno-stimulant activities (5, 6). The principle constituents of the extract are tannins, flavonoids, alkaloids, saponins and phenolic compounds (7). Traditionally in the folk medicine the plant has been used in the treatment of ulcers but no evidence is available that reveal the role of Cow urine piper betel extract in augmenting the peptic ulcers. Hence, the present investigation was aimed to explore the anticonvulsant potential of Cow urine betel vine extract in MES and pentylene tetrazole induced seizure models.

MATERIALS AND METHODS

The leaves of betel vine was collected from the nearby plantations and authenticated by Dr. Muralidhar, Prof and HOD, Department of biotechnology, Dayananda Sagar Institutions. Later leaves were shade dried and coarsely powdered. Diazepam (4mg/kg. b.w) and Pentylene tetrazole (80mg/kg. b.w) was used as standard drug.

Preparation of extract: 200gms of powdered leaf was extracted with fresh cow urine by maceration process for 4 days at 40-450 C, the filtrate was then and subjected for evaporation to remove moisture content in rotary vacuum evaporator. Later the extract was collected and kept in dessicator for further use.

Qualitative phytochemical investigation:

The cow urine betel vine was then subjected to phyto-chemical investigation for the presence of various secondary metabolites as tannins, amino acids, alkaloids, flavonoids, saponins and proteins using standard methods [8, 9, 10].

Experimental animals:

Wistar rats weighing between 200-250gms were procured from the animal house of Dayananda Sagar College of Pharmacy, Bangalore. They were maintained at standard housing condition at the room temperature of 220C (±30C) and relative humidity of 44-56% with light and dark cycles of 10-14hrs respectively [11,12]. The rats were provided with standard rodent pellet diet and the water ad libitium [13]. The study of the protocol was duly approved by Institutional Animal Ethics Committee (CPCSEA, Reg no: DSCP/M.Pharm/ IAEC/106/Pharmacology).

ACUTE TOXICITY STUDIES:

Acute toxicity was carried out as per the CPCSEA and OECD guidelines 425. Group of 6 healthy mice weighing 20-25gms were selected and kept for 3-4hrs fasting with free access to drinking water. Doses were calculated according to the body weight. The test extracts were dissolved in cow urine and administered orally at the starting dose of 2000mg/kg and were kept for observation for any mortality and behavioral changes for 24hrs, further dose was increased up to 5000mg/kg b.w. It was observed that no mortality was observed even at higher dose of the extract and no change in their behavior. As per the OECD guidelines 1/10th and 1/5th of the maximum tolerated dose was selected. Therefore effective therapeutic doses selected were 250 and 500mg/kg b.w.

PROCEDURE:

Maximal Electric Shock Induced convulsion (In-vivo) [14]

In this model, the animals were divided into six groups with six animals in each group. The animals of group I served as solvent control, received distilled water (1 ml/100gm b.w.); group II receive Phenytoin(25 mg/kg b.w.), treated as positive control; III and IV groups treated with *betel vine* leaves extract at the dose of 250mg/kg and 500mg/kg b.w respectively. All the treated groups were administered i.p one hour prior to the electric shock induced in the entire animals passing a current of 50 mA for 0.2 sec duration through electro convulsive meter (Techno India) using ear electrodes. The duration of flexion, extensor, clonus and stupor phase were noted and Percentage of inhibition of seizures relative to controls was calculated.

Pentylenetetrazole – induced seizure model:[15,16]

In this type of seizure model, the animals were divided into six groups with six animals in each group. Group I served as solvent control, received distilled water (1ml/100gm b.w); Group II received Diazepam (2 mg/kg b.w), treated as positive control and III and IV groups treated with betel vine leaves extract at the dose of 250mg/kg and 500mg/kg b.w. respectively. All the treated groups were administered 60min prior to the administration of Pentylenetetrazole (80 mg/kg b.w) by i.p route. The animals were observed for 1 hour by placing in a separate cage. The duration of seizures (tonic, clonic convulsions) were recorded and Percentage of inhibition of seizures relative to controls was calculated.

RESULT AND DISCUSSION

Preliminary phytochemical tests of Piper betel (Betel vine).

In the, preliminary phytochemical studies of Cow urine *Betel vine* extract confirms the presence of flavonoids, Alkaloids, Phenolic compounds, phytosterols and saponins. Hence for the further pharmacological studies the extract was selected.

Phyto-chemical investigation of Cow urine with Betel vine extract.

SL.NO	TYPE OF TEST	Cow urine extract
1	**Test for CARBOHYDRATES**	
	▪ Molisch's test	+
	▪ Fehling's test	+
	▪ Barfoed's test	+
2	**Test for STARCH**	-
3	**Test for PROTEINS and FREE AMINO ACIDS**	
	▪ Million's test	+
	▪ Biuret's test	+
	▪ Ninhydrin's test	+
4	**Test for PHYTOSTEROLS**	+

5	**Test for FIXED OILS and FATS**	
	▪ Spot test	+
	▪ Saponification	+
6	**Test for ALKALOIDS**	
	▪ Mayer's test	+
	▪ Dragendroff's test	+
	▪ Wagner's test	+
	▪ Hager's test	+
7	**Test for GLYCOSIDES**	
	▪ Legal's test	-
	▪ Balget's test	-
	▪ Borntrager's test	-
	▪ Modified borntrager's test	-
8	**Test for FLAVONOIDS**	
	▪ Shinoida's test	+
	▪ Ferric chloride test	+
	▪ Fluorescence test	+
	▪ Reaction with Alkali and Acids	+
	▪ Zinc, HCL reduction test	+
	▪ Lead acetate solution test	+
9	**Test for SAPONINS**	+
10	**Test for COUMARINS**	-
11	**Test for PROTEIN and PHENOLIC COMPOUNDS**	

	▪ Ferric chloride test	+
	▪ Lead acetate test	+
	▪ Gelatin test	+

➢ (+) indicates positive test result, (-) indicates negative test result.

Maximal Electric Shock Induced convulsion (In-vivo)

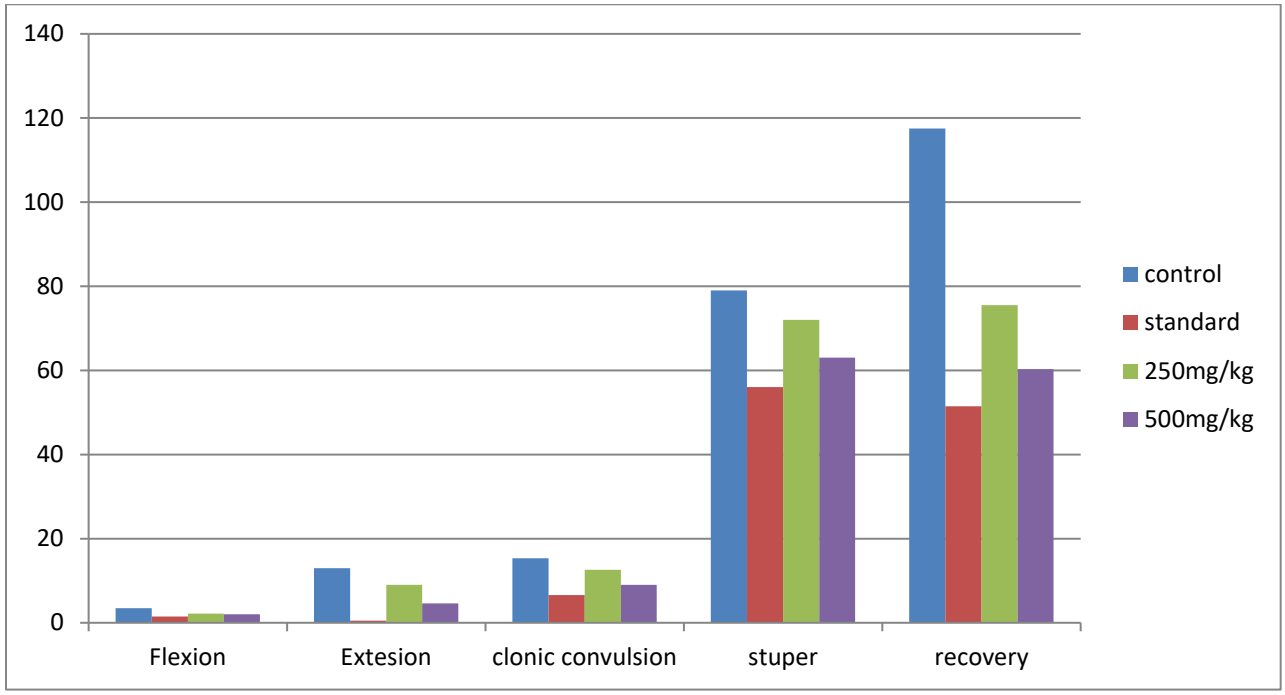
Extract of leaves part exhibited a significant reduction in various phases of epileptic seizures on comparison with the standard drug Phenytoin (25mg/kg b.w). It has been shown a significant reduction in the time required for righting reflex (recovery) in betel vine with cow urine extracts. Prominent anticonvulsant effect has been observed in betel vine extract at (250mg/kg b.w) when compared to the control group. The one way ANOVA analysis of the data observed indicated that in betel vine extract exhibited significant anti-seizure effect against MES induced seizures. Control group animals exhibited hind limb tonic extension of 13±0.6 sec. after the delivery of an electroshock. Betel vine extract at dose of 500 mg/kg body weight shown very less effect on total duration of HLTE (Hind limb tonic extension) phase respectively.

Table No. 1: Effect of *Betel ne with cow urine* on Maximal Electric Shock induced convulsion in mice.

Group	Flexion	Extension	Clonic convulsion	Stuper	Recovery
Control	3.5±0.6	13±0.73	15.3±1.2	79±4.5	117.5±6.7
Standard	1.5±0.2**	0.5±0.12***	6.6±0.7**	56±3.0**	51.5±1.9***
Test 250mg/kg	2.16±0.1*	9.0±0.57***	12.6±0.4*	72±1.1^{ns}	75.5±5.2***
Test 500mg/kg	2.0±0.2**	4.6±0.42**	9.0±.05*	63±1.12**	60.3±2.5***

Note: Data was analysed using one way ANOVA followed by pairwise comparision. Values are expressed as mean ± S.E.M. *n*=6, $^{***}P < 0.001$, $^{**}P< 0.01$and $^{*}P < 0.05$

Figure No.1: Histogram showing the effect of *Betel vine with cow urine* in Maximal Electric Shock Induced convulsion.



Pentylenetetrazole (PTZ) induced convulsions.

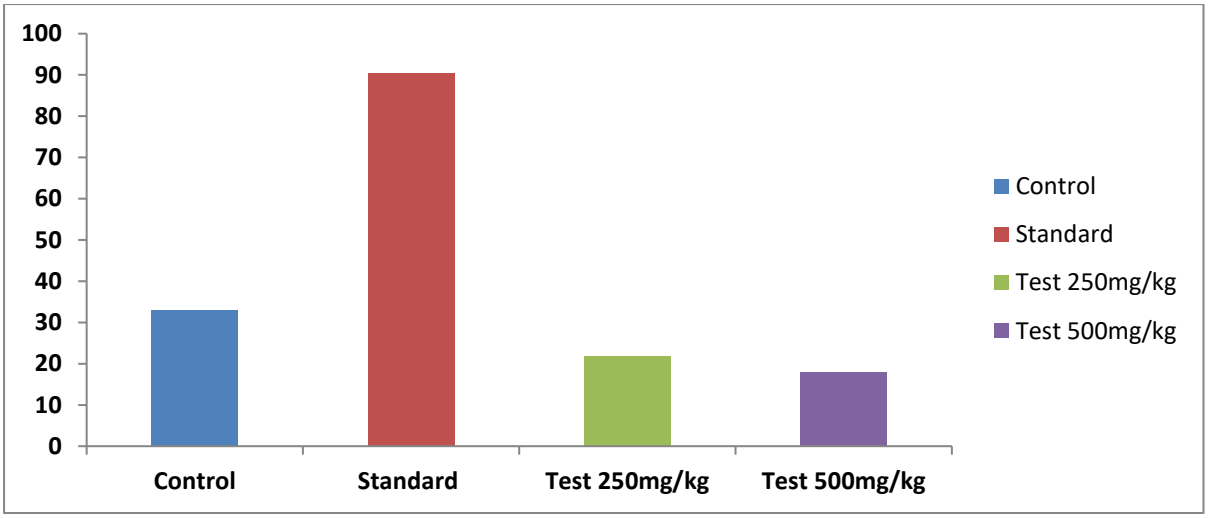
The betel vine extract increased the threshold of PTZ Induced convulsion in mice and offered protection against convulsion. In this model, the onset of convulsion and duration of convulsion in the betel vine extract where completely abolished the convulsion. Whereas extract has also been shown significant anti convulsion activity and also recovery was 100% in all the animals tested. Whereas the standard drug Diazepam (2mg/kg b.w) has been shows completely abolished the convulsion. In other animals of control group, the onset of convulsion and duration of convulsion was more when compared to treated groups of animals and all the animals were recovered and showing no mortality.

Table no. 2: Effect of *Betel vine with cow urine* on Pentylenetetrazole (PTZ) induced convulsion in mice.

Group	Dose (mg/kg)	Onset of convulsion (sec)	No. of convulsion (No.6 animals used)	Mortality (%)	Protection (%)
Control	-	32.9±0.35	6/6	100	0
Standard	4mg/kg	90.3±0.92***	0/6	0	100
Test	250mg/kg	21.9±0.95***	2/6	50	50
Test	500mg/kg	17.8±0.62***	3/6	34	66

Note: Data was analysed using one way ANOVA followed by pairwise comparision.Values are expressed as mean ± S.E.M. *n*=6, $^{***}P < 0.001$, $^{**}P < 0.01$and $^{*}P < 0.05$.

Figure No.2: Histogram showing the effect of *Betel vine with cow urine* on PTZ model Induced convulsion.



DISCUSSION

Medicinal plants are the living resource exhaustible if over used and sustainable if used with care and wisdom. The crude drugs are also used to obtain therapeutically active chemical constituents by specialized methods of extraction, isolation, fractionation and purification are used as phytochemicals for production of herbal medicines.

Reports suggest that the plant contains Alkaloids, Glycosides, Flavonoids, Saponins, Carbohydrates, Fixed oils,Fats Phytosterols, Proteins and Amino acids. The previous studies of the plant claims that it have potential hepatoprotective, Antimicrobial, Neurological activity and anti-oxidant activities.

The present study was undertaken to evaluate the anti-convulsion activity by *Betel vine and cow urine* using experimental models in mice. These activities of the plant have not been reported so far. The phytochemicals are alkaloids, flavonoids, glycosides and phytosterols were identified in the plant.

Maximal Electric Shock Induced convulsion (In-vivo)

Swiss albino mice were used for the screening of anticonvulsant activity in MES induced convulsion. The experiment had been performed where convulsion were induced after 30mins to each group of animals, following the oral route of *Betel vine* with cow urine extract as well as the standard drug Phenytoin.

The results were indicates that the extracts were shown increased the threshold of seizure in the MES test. GABA is the major inhibitory neurotransmitter in the brain while glutamic acid is an excitatory neurotransmitter in the brain. The inhibition of GABA neurotransmitter and the enhancement of the action of glutamic acid have been shown to be the underlying factors in epilepsy. The present study shows that the cow urine betel vine extract protected some of the animals against seizures induced by maximal electroshock. Antiepileptic drugs which inhibit voltage-dependent Na+ channels, such as phenytoin can prevent MES-induced tonic extension [17, 18].

Pentylenetetrazole (PTZ) induced convulsions.

Pentylenetetrazole may elicit seizures by blocking GABA/Cl- channel complex. Picrotoxin induces seizure, by blocking the chloride channels linked to GABA-A receptor. Diazepam, a standard antiepileptic drug is believed to produce their effects by enhancing GABA mediated opening of chloride channel on GABA-A receptor leading to more chloride ion entering the neuron which in turn decreases the neuronal activity in the brain [19]. In the present study diazepam shown to antagonize the seizure induced pentylenetetrazole. The extract was also shown to delay the latency of pentylenetetrazole induced seizures, suggesting that the extract exhibiting anticonvulsant affect, probably by opening the chloride channels associated with GABA receptors.

Thus the results indicate that *Betel vine* with cow urine extracts were effective in absence of seizure as well as tonic clonic seizure (table no- 2). Finally the results from both the model indicate that the plant leaves extract have been found to have broad- spectrum anticonvulsant

activity, however the further research is in progress to isolate the compound responsible for this activity.

CONCLUSION

The above finding thus suggest the anticonvulsant activity of Cow urine betel vine extract, thus support the traditional use of the extract for controlling the convulsions.

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