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An experimental evaluation of *Vishagna* (*Alstonia venenata* R. Br.) a folk medicinal plant for anticonvulsant activity in Swiss Albino Mice

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ABSTRACT

Back ground: Epilepsy describes a condition in which a person has recurrent seizures due to a chronic, underlying process. Globally, it is the third most common neurological disorder. There is still a need for an ideal anticonvulsant agent with broad spectrum activity, rapid onset of action, least side effects, good bio availability and low cost. **Materials and Methods:** *Vishagna* (*Alstonia venenata* R. Br.) belonging to Apocynaceae family is an ethno medicinal plant; the stem bark of the plant is used as antiepileptic drug among tribes. This study aims to experimentally evaluate anticonvulsant activity of the drug in PTZ induced generalized seizures and kindling on Swiss albino mice. The acute oral toxicological evaluation of the drug was conducted prior to the anticonvulsant study. **Result and Conclusion:** Result of the toxicological study reveals that the drug is relatively safe to be used as medicine. The data of the experimental study shows that the drug is moderate to highly effective anticonvulsant in PTZ induced generalized seizures and is mild-moderately helpful in controlling the development of kindled seizures in mice. It also shows that the drug imparts protective action against a sub convulsive dose of PTZ in kindling by inhibiting epileptogenesis and development of potential seizure threshold even when the disease was fully developed.

Key words: *Vishagna, Alstonia venenata R. Br., Anticonvulsant activity, Epilepsy, Folk medicine.*

INTRODUCTION

Medicinal plants have important contributions in the healthcare system of local communities as the main source of medicine for the majority of the rural population. With enormously diversified ethnic groups and rich biological resources, India represents one of the great emporia of ethno botanical wealth.

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These plants are under threat due to deforestation, overgrazing and their reckless utilization. It indicates the urgent need of their conservation.*Vishagna* (*Alstonia venenata* R. Br.) is a small tree, with grayish brown bark belonging to Apocynaceae family, grows in low to mid elevation deciduous forests of peninsular India.[9] It is in practice among *Kurichyars* as anti-venomous, analgesic, anticonvulsant and anti-pyretic drug. Its stem bark and ripe fruits are said to be effective in epilepsy.[1] Till now no work has been carried out to evaluate its anticonvulsant activity.

Epilepsy is not a single entity but an assortment of different seizures types and syndromes originating from several mechanisms that have in common sudden, excessive and synchronous discharge of cerebral neurons.[2] Approximately 10% of the population will have at least one seizure in their lifetime. The current therapeutic treatment of epilepsy with modern antiepileptic drugs is associated with side-effects, dose related and chronic toxicities.

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Considering the above facts experimental study of the ethno botanical drug *Vishagna* (*Alstonia venenata* R. Br.) was conducted to evaluate its anticonvulsant activity in PTZ induced generalized seizures and kindling in Swiss Albino mice.

MATERIALS AND METHODS

Test drug

Stem bark of *Vishagna* (*Alstonia venenata* R. Br.) was collected from forest of Kollam district Kerala and authentication done by K. Gopalakrishna Bhat, (Retd), Professor, Dept. of Botany, Poornaprajna College, Udupi.

Dosage calculation and administration

Test drug[3]: *Vishagna* (*Alstonia venenata* R. Br.) stem bark *Kashaya* was made in the ratio of 1 part drug powder in 16 parts of water and reduced to 1/8. It was administered orally.

Dose of drug was calculated using Paget and Barnes formula.

Formula = Human dose x Body surface area constant of mice x 50

96 ml x 0.0026 x 50 =12.48 ml / Kg body weight

Reference standard drug: Diazepam 5mg tablets were purchased from market. The solution of diazepam was made by dissolving 5mg tablet in 10ml of distilled water. Drug was administered orally.

Mice dose = 8mg / kg body wt.

Drug for Seizure induction: PTZ was used as chemical for seizure induction. Pentamethylene tetrazole (5mg) was procured from HiMedia laboratories Pvt. Ltd, Mumbai, India.

For Generalized PTZ induced seizures : stoke solution of 80mg PTZ in 10 ml distilled water.

Mice dose = 80 mg / kg body weight.

For PTZ induced kindling : stock solution of 40mg PTZ dissolved in 10 ml distilled water.

Mice dose = 40 mg/ kg body weight. Drug is administered Intra peritoneal.

Experimental animals

Active and healthy 36 Swiss Albino mice of either sex weighing 30-40g were selected for both the studies. Mice used for other studies, pregnant and diseased were excluded.

Animal housing

Swiss Albino mice bred in S.D.M. Center for Research in Ayurveda and Allied Science were taken for the study. They were fed with feed of 'Sai Durga Feed and Food, Bangalore' and tap water was given *ad-libitum.* The temperature of 22± 03°C and humidity of 50-70% were maintained and animals were exposed to natural day-night cycles. The rats were kept under observation (acclimatization) for seven days under normal laboratory diet. They were observed for general behavior, weight, per day food and water intake.

The experiment was conducted as per guidelines and prior permission of Institutional Animal Committee (SDMCRA/IAEC/UD/RS-01).

Grouping of animals

Swiss albino mice were made in to 3 groups of 6 rats. The group 1 (control) was treated with distilled water, group 2 (standard) with diazepam and group 3 (test) with *Vishagna* (*Alstonia venenata* R. Br.).

Acute toxicity study of test drug[4]

Acute toxicity test was conducted to determine LD_{50} dose of *Vishagna* (*Alstonia venenata* R. Br.) *Kashaya*. Drug was administered orally at different dose level in 5 different rat in 175mg/kg, 550mg/kg, 2000mg/kg, 2000mg/kg, 2000mg/kg all the animals were observed at ½, 1, 2,3,4, 24 and 48hrs dosing and there after daily once for mortality during the entire period of 14 days study. Maximum tolerated dose was calculated by employing OECD 425 guidelines with AOT software. The LD_{50} value was found to be less than 2000mg/kg.

Anticonvulsant Study[5]

PTZ induced generalized seizure method

Here test drug and standard drug was administered to animals of respective groups orally for 5 consecutive

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days. On the 6th day they were subjected to chemo-convulsions by administering pentylenetetrazole intra peritoneal and watched for the following convulsive profile parameters.

- Latency of onset of seizures
- No of myoclonic jerks
- Duration of myoclonic jerks
- No of straub tail
- No of clonic convulsions
- No of tonic clonic convulsion recurrence
- Duration of recurrence
- Duration of death/live

Abolishing of clonic convulsion was considered as the index of anticonvulsant study. The mean of the readings were calculated compared with that of Control group.

PTZ induced kindling method

Kindling is a model of epilepsy produced by repeated administration of an initially sub convulsive electrical or chemical stimulus that results in an increase in seizure activity. In this model, the effect of drug on both focal and generalized seizure types can be quantitated.

Animals in test and standard group were orally administered with their respective drug for 22 consecutive days. PTZ sub-convulsive dose of 40mg /kg body weight was administered intra peritoneal to all the animals on every other day for 11 days. The groups were observed for parameters about 35 minutes each. Calculations were made on account of the scoring system of Fischer and Kittner-1998.

- Grade 0 - no evidence of convulsive activity
- Grade 1 - ear and facial twitching, head nodding
- Grade 2 - myoclonic jerks
- Grade 3 - forelimb clonuses, fall rearing
- Grade 4 - generalized clonic convulsions, rearing, jumping, falling , loss of righting reflex
- Grade 5 - clonic- tonic convulsions, tonic hind limb extensions.

The scores of test and control groups after mean calculation were compared to Control group.

Statistical calculations

Values of each parameter of all the groups were expressed in MEAN± SEM. The data were analyzed by one- way ANOVA followed by Dunnett's multiple comparisons 't' test as post hoc test. Graph Pad InStat 3 was used for calculations. A level of p=0.05 was considered as statistically significant. Levels of significance were noted and interpreted accordingly.

OBSERVATION AND RESULTS

Generalized seizures

Latency of onset and no of myoclonic jerks (sec)

Table 1: Effect of *Vishagna* on latency of onset and no of myoclonic jerks in PTZ induced convulsions.

Groups	Onset of seizure (in sec)	% change	No of myoclonic jerks	% change
Control (A)	224.16±94.81	-	6.5±1.8	
Standard (B)	232.83±32.2	3.8↑	4.5±1.72	30.7↓
AV(C)	305.5±116.52	36.2↑	7.33±2.12	12.7↑
Data: MEAN±SEM				

Data shows that there was an increase in the duration of latency of onset and no of myoclonic jerks in test group compared to control group. The observed increase was not statistically significant. There was increase in duration of latency of onset and decrease in no of jerks in standard group compared to control. The observed increase and decrease was not statistically significant.

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No of straub tail occurrence and clonic convulsions

Table 2: Effect of *Vishagna* on no of Staub tail occurrence and no of clonic convulsions in PTZ induced convulsions.

Groups	No of straub tail	% change	No of clonic convulsions	% change
Control (A)	1.66±0.21	-	8.83±3.36	
Standard (B)	0.50±0.34*	50↓	0±0*	100↓
AV(C)	0.5±0.34*	50↓	1.16±0.66*	86.8↓
Data: MEAN±SEM, *P<0.05				

Data shows that there was decrease in no of straub tail occurrence and no of clonic convulsions in test group and standard group compared to control group. The observed decrease was found to be statistically significant.

Duration and no of tonic clonic convulsions recurrence

Table 3: Effect of *Vishagna* on duration and no of tonic clonic convulsions recurrence in PTZ induced convulsions.

Groups	No of tonic clonic convulsions	% change	Duration	% change
Control (A)	3.8±0.94	-	72.3±12.49	
Standard (B)	0±0**	100↓	0±0**	100↓
AV(C)	2.66±0.80	166↑	42.5±10.55	412↓
Data: MEAN±SEM, **P<0.01				

Data shows that there was decrease in duration and no of tonic clonic convulsion recurrence in standard group compared to control group. The observed decrease was found to be statistically very significant.

There was increase in no of tonic clonic convulsion recurrence and decrease in duration of tonic clonic convulsions in test group compared to control group. The observed increase was found to be statistically not significant.

Kindling

Total no of grades

Table 4: Effect of *Vishagna* on total grade in PTZ induced convulsion kindling.

Groups	Total no of grades in kindling	% change
Control (A)	289.83±54.46	-
Standard (B)	205.5±32.74	29.09↓
AV(C)	191.33±19.71	33.98↓
Data: MEAN±SEM		

Data shows that there was decrease in total no. of grades in test group compared to control group. The observed decrease was found to be statistically not significant. There was decrease in total no of grades standard group compared to control group. The observed decrease was found to be statistically not significant.

Kindling no of grade 1 (facial twiching / head nodding) and at peak time

Table 6: Effect of *Vishagna* on no. of grade 1 in PTZ induced convulsion kindling

Groups	No of grade 1	% change	At peak	% change
Control (A)	39.83±9.995	-	3.833±1.138	
Standard (B)	117.66±17.150**	195.4↑	16.166±3.429**	321.75↑
AV (C)	76.66±11.918	92.46↑	9.66±1.174	152.02↑
Data: MEAN±SEM, ** P<0.01				

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Data shows that there was increase in no of facial twitching / head nodding in test group compared to control group, even in its peak time. The observed increase was found to be statistically not significant. There was increase in no of facial twitching / head nodding in standard group compared to control group even in its peak time. The observed increase was found to be statistically very significant.

Kindling no. of grade 2 (no of myoclonic jerks) and at peak time

Table 7: Effect of *Vishagna* on no of grade 2 in PTZ induced convulsion kindling.

Groups	No of grade 2	% change	At peak	% change
Control (A)	89.833±21.364	-	22±4.575	
Standard (B)	28.833±3.390*	67.9↓	6.5±0.718**	70.45↓
AV (C)	48.33±8.973	46.2↓	7±1.96**	68.18↓
Data: MEAN±SEM, * P < 0.05, **P<0.01				

Data shows that there was decrease in no of myoclonic jerks in test group compared to control group. The observed decrease was found to be statistically not significant. There was decrease in no of myoclonic jerks in standard group compared to control group. The observed decrease was found to be statistically significant. The observed decrease at the peak time was statistically very significant in both cases.

Kindling no of grade 3 (no of clonic convulsions) and at peak time

Table 8: Effect of *Vishagna* on no. of grade 3 in PTZ induced convulsion kindling

Groups	No of grade 3	% change	At peak	% change
Control(A)	6±1.549		0.5±0.34	
Standard (B)	10.83±5.082	80.5↑	0.830±0.65	66↑
AV (C)	5.5±1.821	8.3↓	0.33±0.33	34↓
Data: MEAN±SEM				

Data shows that there was decrease in no of clonic convulsions in test group compared to control group even at peak time. The observed decrease was found to be statistically not significant. There was increase in no of clonic convulsions in standard group compared to control group even at peak time. The observed increase was found to be statistically not significant.

Kindling no. of grade 4 and at peak (no. of tonic clonic convulsions) and at peak time

Table 9: Effect of *Vishagna* on no. of grade 4 in PTZ induced convulsion kindling

Groups	No of grade 4	% change	At peak	% change
Control (A)	9.833±2.428		2±0.81	
Standard (B)	0±0**	100↓	0±0*	100↓
AV(C)	5±0.774	49.15↓	0.5±0.34	75↓
Data: MEAN±SEM, *P<0.05, ** P<0.01				

Data shows that there was decrease in no. of tonic clonic convulsions in test group compared to control group. The observed decrease was found to be statistically not significant. There were no clonic convulsions in standard group compared to control group. The observation was found to be statistically very significant. At the peak time the observation was found to be statistically significant.

Kindling no. of grade 5 (hind limb extension) and at peak time

Table 10: Effect of *Vishagna* on no. of grade 5 in PTZ induced convulsion kindling

Groups	No of grade 5	% change	At peak	% change
Control (A)	1.33±0.55		0.5±0.34	
Standard (B)	0±0*	100↓	0±0	100↓
AV(C)	0.5±0.22	62.4↓	0.16±0.66	68↓
Data: MEAN±SEM, * P<0.05				

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Data shows that there was decrease in hind limb extension in test group given with *Vishagna* compared to control group, even at peak. The observed decrease was found to be statistically not significant. There was no hind limb extension in standard group compared to control group. The observation was found to be statistically significant. At the peak time it was statistically not significant.

DISCUSSION

Anticonvulsant activity in PTZ induced convulsion in mice

Table 11: Effect of *Vishagna* (test group) and diazepam (standard group) on parameters of PTZ induced convulsions compared to control group

Parameters	Standard group	Test group
Latency of onset of seizure (in sec)	NSI	NSI
Total no of myoclonic jerks	NSI	NSD
No of straub tail occurrence	SD	SD
No of clonic convulsion	SD	SD
No of tonic clonic convulsions recurrence	SD	NSI
Duration of tonic clonic convulsions	SD	NSD

NSD - Non Significant Decrease, NSI - Non Significant Increase, SD - Significant Decrease

Though statistically not significant there is increase in the latency of onset of seizures in case of test drug and standard drug compared to control group. Test group shows 36.2% increase and standard group shows only 3.8% increase.

There is significant decrease in straub tail occurrence and no. of clonic convulsion in test and standard group compared to control group with same percentage increase to that of standard group. There is significant decrease in no. of tonic clonic recurrence in standard group but not in test group compared to control. There is decrease in duration of tonic clonic convulsions in test and standard groups compared to control group but the decrease is not statistically significant.

The data shows that *Vishagna* was effective in controlling all the parameters except that of tonic-clonic convulsion even though statistically not significant and shows very effective and statistically significant control of some of the parameters. This indicates that the drug is moderate to highly effective anticonvulsant.

Anticonvulsant activity in PTZ induced kindling in mice

Table 12: Effect of *Vishagna* (test group) and diazepam (standard group) on parameters of PTZ induced convulsions kindling compared to control group

Parameters	Standard group		Test group	
	Total	At peak	Total	At peak
Total grades	NSD	-	NSD	-
Grade 1 - Facial twitching	SI	SI	NSI	NSI
Grade 2 - Myoclonic jerk	SD	SD	NSD	SD
Grade 3 - No of clonic convulsions	NSI	NSI	NSD	NSD
Grade 4 - No of tonic clonic convulsion	SD	SD	NSD	NSD
Grade 5 - Hind limb extension	SD	NSD	NSD	NSD

Control group mice showed all grades of convulsions in a severe form and peak was attained by 4th day. Except 2, all mice in the control group were dead by

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14th day. Survival rate of standard group was 100% till the last day of kindling. Test group mice showed better survival rate. All mice survived till 20th day in test group. Test group mice were active till last date of kindling.

Total no of grades decreased in both test and standard groups compared to control but the decrease is not statistically significant. Standard group showed statistically significant decrease in grade 2, 4, 5 and there were absence of tonic-clonic convulsions in the group.

Test group showed decrease in all grades like myoclonic jerk, clonic convulsion, tonic-clonic convulsion and hind limb extension even though the decrease was not statistically significant compared to control group.

At the peak time (10-15 min) of kindling statistically significant decrease in grade 2 and 4 is observed in standard group compared to control group. In test group statistically significant decrease is observed in grade 2 - myoclonic jerk and decrease in all other grades are also observed even though statistically not significant when compared to control group.

By studying observations of kindling we can assume that the drug is mild-moderately helpful in controlling the development of kindled seizures in mice. By the lesser intensity of grades in test group we can access that the drug imparts protective action against a sub convulsive dose of PTZ in kindling by inhibiting epileptogenesis and development of potential seizure threshold even when the disease was fully developed. The active state of mice in test group showers light on non-production of psychological or cognitive side effects by long term use of drug unlike the commonly used AEDs with sedation effect or depression.

Probable mode of action

Glutamatergic synaptic transmission plays an imperative role in the weakening of GAB Aergic inhibitory processes induced by PTZ. The test drug shows moderate to high anticonvulsant activity by significant decrease in no. of straub tail and no. of clonic convulsions. Here the drug action may be by potentiating these GABAergic neurotransmission or may be by acting as voltage gated Na^+ or Ca^{2+} channel blocker leading to stabilization of hyper excited neural membranes that is by direct or indirect action on ions. The exact mechanism of counter action of test drug in PTZ induced seizures needs to be elucidated.

The major active principles of the stem bark exhibits reserpine like profile of activity like sedation, ptosis, and reduction in motor activity.

Tikta Rasa is *Medhya* according to Astanga Hrudaya and *Moorcha Prasamana* according to *Sushruta Acharya*. *Medhya Dravya* helps in preventing attainment of *Upahata Chetas* in *Apasmara* (epilepsy) which is one of the primordial causative factors told in *Samprapti* (pathophysiology).

Fig. 1: *Alstonia venenata* R. Br.

Fig. 2: *A. venenata* flower

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Fig. 3: *A. venenata* stem

Fig. 4: *A. venenata* Stem bark

Fig. 5: Clonic convulsion

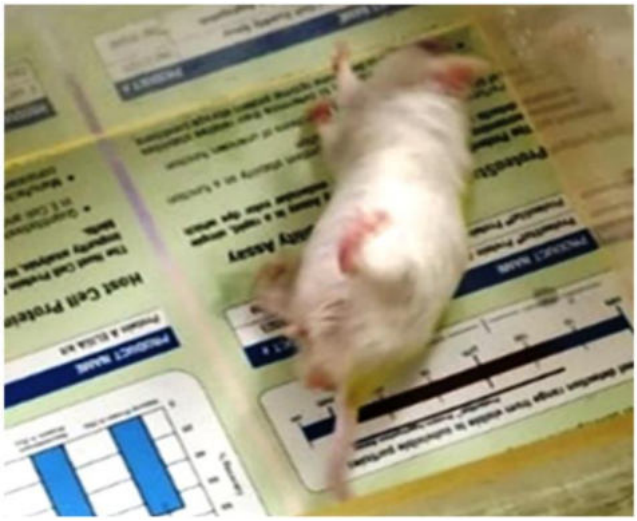
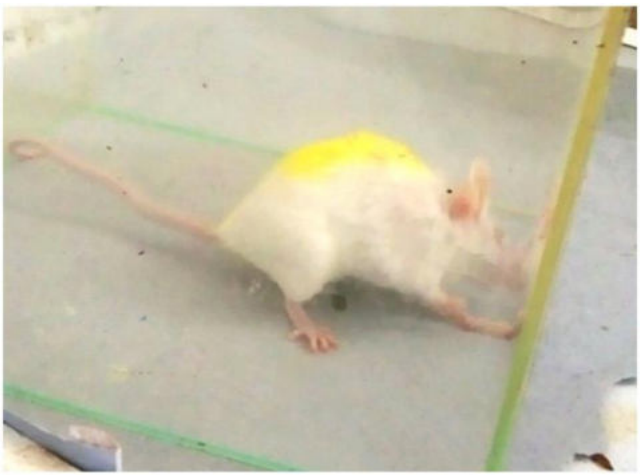


Fig. 6: Myoclonic jerk



CONCLUSION

Alstonia venenata R. Br. is extensively used in epileptic treatment among tribes. To ascertain the anti-epileptic activity of the drug experimental study was carried out in 2 models; PTZ Induced generalized seizures and PTZ Induced kindling in Swiss Albino mice. The results can be interrupted as follows. The data shows that *Vishagna* was effective in controlling all the parameters except that of tonic-clonic convulsion and shows very effective and statistically significant control of the parameters like no. of straub tail occurrence and no. of clonic convulsions. This indicates that the drug is moderate to highly effective anticonvulsant. By studying observations of kindling we can assume that the drug is mild-moderately helpful in controlling the development of kindled seizures in mice. It also shows that the drug imparts protective action against a sub convulsive dose of PTZ in kindling by inhibiting epileptogenesis and development of potential seizure threshold even when the disease was fully developed.

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