

**ANTIEPILEPTIC ACTIVITY OF MURRAYA KOENIGII LEAF AQUEOUS EXTRACT IN PENTYLENETETRAZOLE AND STRYCHNINE INDUCED CONVULSIONS IN RATS AND MICE**

Vanna Manjusha\*, Gella Suneel

Department of Pharmacology, Malla Reddy College of Pharmacy, Hyderabad, India

**\*Corresponding author e-mail:** manjuvanna@gmail.com**Received on:** 06-12-2015; **Revised on:** 15-12-2015; **Accepted on:** 24-12-2015**ABSTRACT**

The aim of present study was to investigate antiepileptic activity of aqueous extract of *Murraya koenigii* (AEMK) on pentylenetetrazole (PTZ) and strychnine (STR) induced convulsions in mice and rats respectively. 24 male *Swiss mice* and 24 male *Wistar rats* were divided into four groups of six animals each as I, II, III and IV which were treated with 0.9 % saline (10 ml/kg, p.o.), Diazepam (4 mg/kg, i.p.), AEMK (200 mg/kg, p.o), AEMK treated (400 mg/kg, p.o). All mice were treated with PTZ (75 mg/kg, i.p.) and all rats with Strychnine (2 mg/kg, i.p.), 30 min after i.p administration of diazepam and 60 min after oral administration of saline and extract doses. Onset and duration of convulsions, percentage protection, Severity score and mortality rate in rats and mice were recorded. Statistical analysis was carried out by ANOVA followed by Barlett's test. In present study AEMK decreased severity of convulsions, increased percentage protection, decreased mortality rate and exhibited significant antiepileptic activity.

**Key words:** Epilepsy, Pentylenetetrazole, Strychnine, *Murraya koenigii*.**INTRODUCTION**

Epilepsy is a common neurological disorder characterized by an imbalance between excitatory and inhibitory neurotransmission. The incidence rate varies from 38 to 49.3 per 100,000 population per year from two community-based studies in India.<sup>[1]</sup> Approximately 20% of population suffering from epilepsy is of child bearing age. Several antiepileptic drugs (AEDs) employed for treating epilepsy are causing teratogenic effects, for example phenytoin causes fetal hydantoin syndrome, valproic acid and carbamazepine causes neural tube defects, phenobarbital causes congenital heart defects etc., Considering these, various traditional plants have been tried in past for their antiepileptic properties<sup>[2,3,4,5]</sup>

*Murraya koenigii* is a small tree belonging to family Rutaceae.<sup>[6]</sup> The leaves are pinnate and are highly aromatic. The flowers are small, white, and fragrant.

The small black shiny berries are edible. Several studies suggested the anti-inflammatory, analgesic<sup>[7]</sup>, anti-obesity<sup>[8]</sup>, anti-diabetic<sup>[9]</sup>, anti-diarrheal<sup>[10]</sup>, anti-cancer<sup>[11, 12, 13, 14]</sup> and immunomodulatory<sup>[15]</sup> activities of *Murraya koenigii*. It possesses excellent anti-oxidant activity and it is a known fact that anti-oxidants are proven neuroprotectors<sup>[16]</sup>. In this context, the present study aims to evaluate the anti-epileptic activity of *M. koenigii*.

**MATERIALS AND METHODS**

**Plant material:** The fresh leaves of *Murraya koenigii* were collected from its natural habitat in Hyderabad in Telangana, India. The plant was authenticated by Botany Department and a voucher specimen (No. BSI/DRC/2014-15/Tech/654) was deposited at Herbarium, Department of Botany, Attapur, Hyderabad, India.

**Drugs and chemicals:** PTZ (Sigma-aldrich, bangalore, India) and STR (Sigma-aldrich, Bangalore, Sigma-aldrich) were procured from Sai Krishna enterprises, Hyderabad. Diazepam (CALMPOSE, Ranbaxy laboratories, Delhi, India) used as standard was procured from local medical shop, Hyderabad. All chemicals used were of analytical grade.

**Preparation of extract:** The fresh leaves of *Murraya koenigii* were dried under shade. The dried leaves were made into coarse powder which was passed through a 60 No. mesh sieve, then subjected to extraction at 60°C temperature by soxhlet's extractor using distilled water as a solvent (100 g powder with 200 ml solvent). The extraction was performed for 18 h. The extract was concentrated by evaporation at room temperature, stored in a dessicator and used for study. The percent yield for AEMK was found to be 7.4% w/w.

**Qualitative analysis:** The extract was subjected to phytochemical screening by using different qualitative tests.<sup>[17]</sup>

**Experimental animals:** Healthy male *Swiss mice* (25-30 g) and male *Wistar rats* (200-250g) were used for the experiment. The animals were procured from National Institute of Nutrition (NIN), Hyderabad. They were housed in polypropylene cages with paddy husk as bedding and maintained under standard conditions (Temp 22±2°C, Relative humidity 60±5% and 12 h light/dark cycle). They had free access to standard pellet diet and water *ad libitum*. All the animals were allowed to acclimatize one week before use. All experimental protocols were in compliance with the Institutional Animal Ethics Committee (IAEC) for the care and use of laboratory animals and were approved by the Department of Pharmacology, Malla Reddy College of Pharmacy Ethics committee. (Approval no: MRCP/CPCSCEA/12172008).

**Pentylentetrazole (PTZ) induced convulsions in mice** <sup>[18]</sup>: PTZ induces convulsions by inhibiting Gamma amino butyric acid (GABA) synthesis, which is an inhibitory neurotransmitter. PTZ (75 mg/kg, i.p.) was administered one hour after oral treatment with vehicle, standard or test extract. All the doses were selected from previous studies.

**Experimental design:** Twenty four male *Swiss mice* were divided into four groups and were treated as follows:

Group I : Disease control (0.9 % saline p.o.+ PTZ 75 mg/kg, i.p.)

Group II : Standard treated (Diazepam 4 mg/kg, i.p. + PTZ 75 mg/kg, i.p.)

Group III : Aqueous extract of *Murraya koenigii* treated (200 mg/kg. p.o + PTZ 75 mg/kg, i.p.)

Group III : Aqueous extract of *Murraya koenigii* treated (400 mg/kg. p.o.+ PTZ 75 mg/kg, i.p.)

I, II, III and IV groups were treated with, saline, diazepam, AEMK(200mg/kg) and AEMK(400mg/kg) respectively. All groups were treated with PTZ (75 mg/kg, i.p.), 30 min after i.p administration of diazepam and 60 min after oral administration of saline and extract doses. The onset and duration of convulsions, percentage protection, mortality rate and severity score of seizures (Table 3) were recorded.

**Strychnine (STR) induced convulsions in mice** <sup>[18]</sup>: Strychnine induces convulsions by acting as competitive glycine antagonist. Convulsions were induced by administering STR (2 mg/kg, i.p.), 1 hour after vehicle, standard and test extract treatment.

**Experimental design:** Twenty four male *Wistar rats* were divided into four groups and were treated as follows: Group I: Disease control (0.9 % saline p.o.+ STR (2mg/kg, i.p.)

Group II: Standard treated (Diazepam 4 mg/kg, i.p. + STR (2mg/kg, i.p.)

Group III: Aqueous extract of *Murraya koenigii* treated (200 mg/kg. p.o + STR (2mg/kg, i.p.)

Group III: Aqueous extract of *Murraya koenigii* treated (400 mg/kg. p.o. + STR (2mg/kg, i.p.)

I, II, III, IV and IV groups were treated with saline, diazepam, AEMK (200mg/kg) and AEMK (400mg/kg) respectively. All groups were treated with Strychnine (2 mg/kg, i.p.), 30 min after i.p administration of diazepam and 60 min after oral administration of saline and extract doses. The onset and duration of convulsions, percentage protection and mortality rate were recorded.

#### STATISTICAL ANALYSIS

The data were analyzed by using one-way analysis of variance (ANOVA), followed by Barlett's test. All values were represented as mean ± SEM. P<0.0001 was considered as statistically significant.

#### RESULTS AND DISCUSSION

**Qualitative analysis:** The preliminary phytochemical screening of AEMK showed the presence of sterols, alkaloids, carbohydrates, phenolic compounds, saponins, triterpenes, volatile oil, proteins and amino acids. (Table 1)

#### Antiepileptic activity:

**PTZ induced seizures in mice:** AEMK exhibited significant anti-epileptic activity against PTZ induced seizures in mice at both 200 mg/kg and 400 mg/kg treated groups compared to disease control. The activity was found to be more in 400 mg/kg AEMK treated group ( $P < 0.0001$ ) compared to 200 mg/kg treated group. (Table 2). The severity score was found to be less in standard and test- treated groups compared to disease control. (Table 3, Fig 1)

**STR induced seizures:** AEMK exhibited significant anti-epileptic activity against STR induced seizures in rats at both 200 and 400 mg/kg doses and the activity was found to be more in 400 mg/kg AEMK treated group compared to 200 mg/kg treated group ( $P < 0.0001$ ). (Table 4)

## DISCUSSION

### PTZ – induced convulsions in mice <sup>[18, 19]</sup>:

The ability of an agent to prevent or delay the onset of convulsions induced by PTZ indicates its anticonvulsant activity. In this study, AEMK exhibited significant anti convulsant effect against PTZ and Strychnine induced seizures by delaying onset of convulsions. It also caused profound increase in duration of convulsions. Diazepam was used in this study as a standard. Anticonvulsant agent showed significant activity by delaying the onset of convulsions and increasing duration of convulsions

PTZ acts as a potent convulsant by inhibiting activity of GABA at GABA<sub>A</sub> receptors. Epileptic drugs such as Diazepam and phenobarbitone are thought to produce their effects by enhancing GABA-mediated inhibition. Seizures induced by PTZ are also blocked by drugs such as ethosuximide, by reducing T-type Ca<sup>2+</sup> currents. Activation of N-methyl –D- Aspartate (NMDA) receptor system is also involved in initiation and propagation of PTZ-induced seizures. Seizures induced by PTZ are decreased by drugs such as felbamate by blocking glutaminergic excitation mediated by NMDA

receptor. Since the extract delayed the occurrence and decreased the duration of convulsions induced by PTZ, it is possible that the anticonvulsant effect of AEMK might be due to any one of the above mentioned mechanisms, which is not tested in the study. There was decrease in the mortality and severity score in extract treated (200 and 400mg/kg) groups when compared to disease control.

**Strychnine (STR) induced convulsions in mice <sup>[18, 19, 20]</sup>:** Strychnine acts as a potent convulsant and the convulsant action of strychnine is due to interference with post synaptic inhibition that is mediated by Glycine. It acts as a selective competitive antagonist to block the inhibitory effect of glycine at all glycine receptors. Treatment with AEMK significantly decreased the severity of convulsions and increased the duration of convulsions. There was decrease in the mortality in extract treated (200 and 400mg/kg) groups when compared to disease control.

The present study revealed that aqueous extract of *Murraya koenigii* exhibited significant anticonvulsant effect by delaying onset and increasing duration of convulsions and reducing mortality in mice and rats against PTZ and STR induced seizures respectively. Thus *Murraya Koenigii* (leaves) may be considered as a valuable plant in both ayurvedic and modern drug development areas because of its versatile medicinal uses. The present work did not include the identification of active principle and its mechanism of action. Therefore further experimental analysis should be carried out for a definitive conclusion.

## ACKNOWLEDGEMENTS

The authors are thankful to the management of Malla Reddy College of Pharmacy, Secunderabad for providing necessary laboratory facilities.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

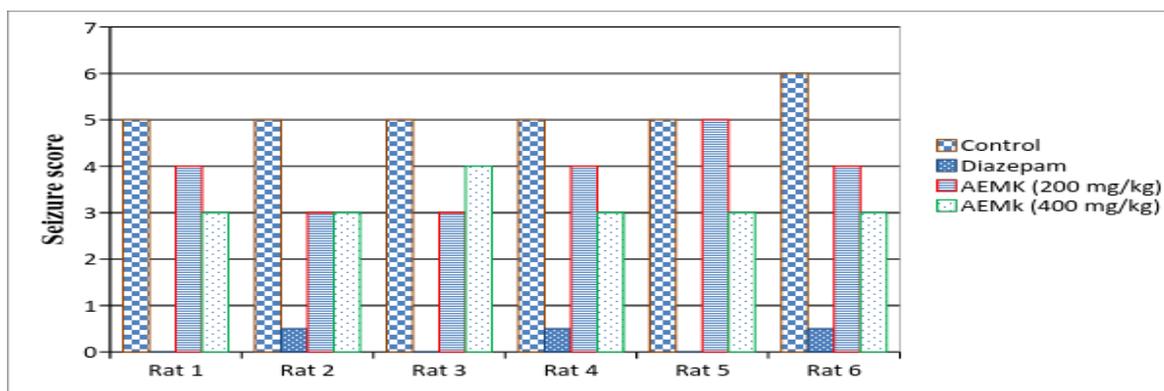


Fig 1: Severity of seizure score in pentylenetetrazole-induced convulsions in mice

**Table 1: Phytochemical screening of *Murraya koenigii***

Chemical Tests	Results
Carbohydrates	+
Protein	+
Phytosterols	+
Tannin	+
Gum/mucilage	-
Flavonoids	+
Volatile oil	+
Anthroquinone Glycoside	+
Alkaloid	+
Saponins	+

+ represents presence  
- represents absence

**Table 2: Effect of AEMK on PTZ - induced seizures in mice**

Treatment	Onset of convulsions(min)	No. Of animals Protected/used	% Protection
PTZ + normal saline	0.2700±0.04879	0/6	0%
Diazepam+PTZ	2.593±0.4092*	4/6	66.7%
AEMK(200mg/kg)+PTZ	0.6080±0.02083 <sup>†</sup>	1/6	18%
AEMK(400mg/kg)+PTZ	0.8900±0.01949 <sup>††</sup>	3/6	50%

Statistical significant test for comparison was done by ANOVA followed by Barlett's test

\*P<0.01, <sup>†</sup>p<0.05 significant when compared to disease control

<sup>††</sup>P<0.0001 compared to AEMK (200mg/kg)

**Table 3: Severity of seizure score in PTZ test**

Score	Changes observed
0	No behavioural abnormalities
0.5	Atypical behavior
1	Isolated myoclonic jerks, ear and facial twitchings
2	Atypical minimal seizures, convulsive wave throughout the body
3	Fully developed minimal seizures, clonus of head, muscles and forelimb, righting reflex present
4	Major seizures, generalized without tonic phase
5	GTCS beginning with running

**Table 4: Effect of AEMK on STR - induced seizures in rats**

Treatment	Onset of convulsions (min)	No. Of animals Protected/used	% protection
Disease control	0.8420±0.1874	0/6	0%
Diazepam+STR	4.524±0.3243*	5/6	83%
AEMK(200mg/kg)+STR	1.936±0.2021 <sup>†</sup>	1/6	16%
AEMK(200mg/kg)+STR	4.900±0.6694 <sup>††</sup>	3/6	50%

Statistical significant test for comparison was done by ANOVA followed by Barlett's test

\*P<0.01, <sup>†</sup>p<0.05 significant when compared to control

<sup>††</sup>P<0.0001 compared to AEMK (200mg/kg)

## REFERENCES

1. Spinella M, Lukasiuk. Herbal medicines and Epilepsy: The potential for benefit and Adverse Effects. *Epilepsy and Behaviour*, 2001; 2: 524-32.
2. Agosta, William C. Medicines and Drugs from Plants. *Journal of Chemical Education*, 1997; 74: 857.
3. Cragg Gm, Newman DJ, Snader KM. Natural products in drugs discovery and development. *Journal of Natural Products*, 1997; 60: 52-60.
4. Cox PA, Balick MJ. The ethno botanical approach to drug discovery. *Scientific American*, 1991; 270: 82-87.
5. Loscher W, Mody I, Potschka H, Prince D. Current status and future directions in the pharmacotherapy of epilepsy. *Trends in Pharmacological Sciences*, 2002; 23:113-18.
6. Raza M, Shaheen, Choudary MI, Sombati S, Rafiq A, Suria A, Rahman A, DeLorenzo, R.J. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from *denudatum*. *Journal of Ethnopharmacology*, 2001;78:73-78.
7. Gupta S et al. Leaf's extract of *murraya koenigii* linn for anti-inflammatory and analgesic activity in animal models. *J Adv Pharm Technol Res*, 2010; 1:68-77.
8. Bonde SD, Nemade LS, Patel MR, Patel AA.. *Murraya koenigii* (Curry leaf): Ethnobotany, photochemistry and pharmacology - A review. *Int J Pharm Phytopharmacology Res*, 2011; 1:23-27.
9. Birari R, Javia V, Bhutani KK. Antiobesity and lipid lowering effects of *Murraya koenigii* (L.) Spreng leaves extracts and mahanimbine on high fat diet induced obese rats. *Fitoterapia*, 2010; 81:1129-33.
10. Kesari AN, Kesari S, Singh SK, Gupta RK, Watal G. Studies on the glycemic and lipidemic effect of *Murraya koenigii* in experimental animals. *J Ethnopharmacology*, 2007; 112:305-11.
11. Mandal S, Nayak A, Kar M, Banerjee SK, Das A, Upadhyay SN. Antidiarrhoeal activity of carbazole alkaloids from *Murraya koenigii* Spreng (Rutaceae) seeds. *Fitoterapia*, 2010; 81:72-74
12. Syam S, Abdul AB, Sukari MA, Mohan S, Abdelwahab SI, Wah TS. The growth suppressing effects of girinimbine on HepG2 involve induction of apoptosis and cell cycle arrest. *Molecules*, 2011; 16:7155-70.
13. Bhattacharya K, Samanta SK, Tripathi R, Mallick A, Chandra S, Pal BC. Apoptotic effects of mahanine on human leukemic cells are mediated through crosstalk between Apo-1/Fas signaling and the Bid protein and via mitochondrial pathways. *Biochem Pharmacology*, 2010; 79:361-72.
14. Ito C, Itoigawa M, Macao K, Murata T, Tsuboi M, Kaneda N, and Furukawa H. Induction of apoptosis by carbazole alkaloids isolated from *Murraya koenigii*. *Phytomedicine*, 2006; 3:359-65.
15. Roy MK, Thalang VN, Trakoontivakorn G, Nakahara K. Mechanism of mahanine-induced apoptosis in human leukemia cells (HL-60). *Biochem Pharmacology*, 2004; 6:41-51.
16. Premanad R, Ganesh T. Neuroprotective effects of *Abrus precatorius* Linn. Aerial extract on hypoxic neurotoxicity induced rats. *International journal of chemical and pharmaceutical sciences*, 2010;1: 9-15.
17. Khandelwal K R. Practical pharmacognosy. Techniques and experiments. 19<sup>th</sup> ed., Pune; Nirali Prakashan: 2008, pp. 45-59.
18. Shah AS, Wakade AS, Juvekar AR. Immunomodulatory activity of methanolic extract of *Murraya koenigii* (L) Spreng. Leaves. *Indian J Exp Biol* 2008; 4:505-09.
19. Tejas Gosavi P , Amit Kandhare D, Pinaki Ghosh, Subhash Bodhankar. Anticonvulsant activity of Argemone metallicum, a homeopathic preparation. *Der Pharmacia Lettre*, 2012; 4:626-37.
20. Tamboli AM, Rub RA, Ghosh P, Bodhankar SL. Antiepileptic activity of lobeline isolated from the leaf of *Lobelia nicotianaefolia* and its effect on brain GABA level in mice. *Asian pac J Trop Biomed*, 2012; 2:537-42.